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# Mechanical Processing of European Green Crab (*Carcinus maenas*), the Development of a Value-Added Product and the Use of Restructuring Additives to Increase the Functional Properties of Green Crab Patties

Joseph A. Galetti

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**MECHANICAL PROCESSING OF EUROPEAN GREEN CRAB (*CARCINUS MAENAS*), THE  
DEVELOPMENT OF A VALUE-ADDED PRODUCT AND THE USE OF RESTRUCTURING  
ADDITIVES TO INCREASE THE FUNCTIONAL PROPERTIES OF GREEN CRAB PATTIES**

By

Joseph A. Galetti

B.S. Johnson & Wales University, 2006

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Food Science and Human Nutrition)

The Graduate School

The University of Maine

December, 2010

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Thesis Advisors: Dr. Denise Skonberg and Dr. Beth Calder

An Abstract of the Thesis Presented  
in Partial Fulfillment of the Requirements for the  
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December, 2010

The European Green Crab (*Carcinus maenas*) has proven to be a successful invasive predator and has potential to be a valuable food source for human consumption. Due to the small size of green crab, hand picking commercial operations are not feasible. The objectives of this research were to develop uses for this undervalued resource by examining mechanical separation techniques of mince meat; developing a consumer acceptable mince meat product and exploring the functionality of green crab mince meat with additions of restructuring additives.

The first part of this research was designed to determine how boil versus steam thermal processing, large (>55 mm carapace width) versus small (<55 mm carapace width) crab size, and 0.000 versus 0.180 breaker bar setting on the mechanical separator affected green crab mince meat yield and quality. Mechanical processing of green crabs resulted in high mince yield ( $\bar{x}$  = 49.2 %) and low crude lipid content (<1.9



%) which indicates its potential to be economically viable in processing operations and to retain its quality during extended periods of frozen storage. Interestingly, small crabs contained significantly ( $p \leq 0.05$ ) more lipid content than large crabs. Proximate analyses showed green crab mince to contain ~10.4 % protein and ~5.1 % ash. Low total volatile base nitrogen (TVBN) and aerobic plate count (APC) values indicate that green crab mince was of good microbial quality. Overall results indicate that small green crabs which were mechanically processed at a 0.000 breaker bar setting, regardless of thermal treatment resulted in the highest mince yield and were easiest to process.

The second part of this research focused on the development of a consumer acceptable empanada containing green crab mince and the effects of 30, 50 and 70 % green crab mince by filling weight. Statistical analysis indicated that empanadas containing 30 and 50 % mince had significantly ( $p \leq 0.05$ ) higher attribute ratings of filling appearance, flavor and overall acceptability compared to the empanadas with 70 % mince. All of the attribute scores of the 30 and 50 % empanadas approached 'like moderately' which indicates potential for this novel product. Panelist comments indicated that a sauce may be beneficial in enhancing the moistness and crab flavor of the empanadas and many of the panelists would 'probably or definitely buy' green crab and vegetable empanadas if they were available to them locally.

The third part of this study determined how additions of transglutaminase, isolated soy protein, dried egg white and their combinations affected the textural profile, color ( $L^*a^*b^*$ ), cook yield, water-holding capacity and proximate composition of green crab mince patties. Results indicated a significant ( $p \leq 0.05$ ) decrease in moisture

content and patty hardness, and a non-significant decrease in yield as additions of transglutaminase increased. Additions of isolated soy protein and dried egg white resulted in more significant changes in functionality than transglutaminase alone, although the combination of 5 % isolated soy protein and 2 % transglutaminase appeared to change the functional properties of green crab mince patties most significantly based on textural and proximate analyses.

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## CHAPTER 1. INTRODUCTION

### History and Habits of the European Green Crab

The European green crab (*Carcinus maenas*) is native to most of Western Europe and is found in the Baltic and North Sea, as well as the Bay of Biscay, and as far south as Morocco in the Atlantic Ocean. *Carcinus maenas* is not found in the Mediterranean Sea; green crab located in the Mediterranean Sea is referred to scientifically as *Carcinus aestuari*. There is a long standing debate in the ecological community as to whether there is genetic variability between the two species. Evidence from studies indicates differences in male swimmeret structure, length-to-width ratio and setae distribution of green crabs from opposing regions (Yamada and Hauck, 2001; Brian et al., 2006). These studies indicate that green crabs from Mediterranean and Atlantic regions are of two distinct species: *Carcinus maenas* Linnaeus and *Carcinus aestuari* Nardo. In contrast, other research has shown overlap among green crab morphological features between Mediterranean and Atlantic regions which mean green crabs cannot be designated to a region based on shape (Clark et al., 2001; Brian et al., 2006).

In Europe, the green crab is commonly referred to as the shore crab, most likely receiving its name because it lives close to coastal shore lines. The green crab was believed to be introduced to the United States Atlantic coast during the early 19<sup>th</sup> century. Within the next hundred years, the green crab had spread into coastal niches originating from Cape Cod and spreading northerly up to the coasts of Maine and Nova Scotia. Currently, it can be found in these locations and as far south as the Carolinas.

The first documented report of green crab along the U.S. West Coast was in 1989 in Estero Americano near San Francisco Bay. Yamada (2001) reported that in 1995, green crabs were found in Bodega Bay, about 75 miles north of San Francisco. Green crab can now be found on the U.S. Pacific Coast ranging from Baja, California up through Canada and along the Southern Alaskan Coast.

The European Green Crab (*Carcinus maenas*) has proven to be a successful invasive predator having found niches in all of the continents with the exception of Antarctica. Green crabs have a very high reproductive output with females able to spawn up to 185,000 eggs at a time under optimal conditions, although only a very small percent survive to adulthood. Other factors that contribute to green crabs invasive nature include: a planktonic larval phase, a tolerance to a wide range of temperatures and salinities and their ability to thrive in close proximities to one another (Crothers, 1968; Young et al., 1999; Naczk et al., 2004). The major regions of the world that have had the most green crab impact include the coasts of: southern Australia, New Zealand, Japan, South America, South Africa, and both coasts of North America. Even in the green crab's native waters of Western Europe, it is often perceived as an invader due to their detrimental effects on commercially viable aquatic organisms.

The green crab can tolerate temperatures ranging from 0° C to 33° C and can survive exposure to air for at least 10 days (Crothers, 1968). These attributes allow green crabs to travel on an airplane in marine shipping materials or in a boat, thus introducing it to new coastal marine systems. The spread of the green crab most likely occurs during the larval stage. Research has suggested that green crab larvae travel in

the ballast water of ships which spreads green crabs quickly, as some ships are able to cross the Atlantic in as little as three days. Adult crabs have been found in packaging materials used to transport New England bait worms and lobsters. Some researchers have speculated that green crabs are able to be transported in the seawater pipes of ships or released into the environment as discarded research material (Cohen et al., 1995; Carlton and Cohen, 2003). Once the green crab has made its transcontinental journey, it is then able to spread along the coast by ocean currents while in the larval stage. These ocean currents are expanding the range of the green crab northward along the U.S. northeastern coast and further up into Canada.

Gut content studies have concluded that green crabs consume a wide array of aquatic organisms, including oysters, mussels, snails, clams, and other crabs. Despite their relatively small size of less than 95 mm in carapace width, green crabs are able to open bivalves with relatively thick shells (Grosholz et al., 2000; Naczk et al., 2004). In addition to feeding on bivalves and other shellfish, green crabs act as competitors or predators towards the American lobster (*Homarus americanus*) and rock crab (*Cancer irroratus*) which are two commercially important crustaceans (Gillis et al., 2000; Naczk et al., 2004). The fact that green crab feeds on bivalves has a severe impact on local ecosystems. Migrating birds are also affected when there is a lack of bivalves to feed on, subsequently affecting ecosystems non-local to the green crab.

## The Crab Industry

Crabs are classified scientifically as decapod crustaceans of the Brachyura infraorder with almost 7,000 species known. All crabs have a small abdomen which is generally tucked under their thorax, and the term Brachyura literally translates to “short tail” from Greek. They are found in all of the world’s oceans with approximately 850 freshwater or semi-terrestrial known species. Crabs can vary in size from only a few millimeters in width to four meters in leg span, as seen with the Japanese spider crab.

Some commercially viable crab species found in U.S. waters include: the Dungeness crab (*Cancer magister*), the blue crab (*Callinectes sapidus*), the stone crab (*Menippe mercenaria*), the Jonah crab (*Cancer borealis*), the king crab (several species in the family *Paralithodes*), the snow or tanner crab (*Chionocetes bairdi*, *C. opilio*, or *C. tanneri*) and the red or golden crab (*Geryon quinquedens* or *G. fenneri*) (Osterling, 2000). Other notable species found on a worldwide basis include; the edible crab (*Cancer pagurus*), the sand crab (*Portunus pelagicus*), the Gazami crab (*Portunus trituberculatus*) and the mangrove or mud crab (*Scylla serrata*).

In 2008, the U.S. National Marine Fisheries Service estimated that only 76.3 metric tons of green crabs were harvested which equates to a value of approximately \$163,883 (NMFS, 2008). In comparison, other U.S. crab fisheries landed 69,373.2 metric tons (blue crab), 22,654.5 metric tons (Dungeness crab), 12,341.4 metric tons (king crab) and 28,323.6 metric tons (snow crab). Currently, the value of Jonah crab mince is approximately \$2.10 per pound in Portland, Maine (Wuerthner, 2010).

Most commercially harvested crabs are caught in steel traps or “pots” usually baited with high-fat fish. On the U.S. Atlantic Coast, common baits used to catch blue crab include: eel, herring, mackerel, menhaden and even chicken. After the crabs are caught, they are screened for mortality, sorted by size and placed in plastic totes or bushel baskets (Senkel et al., 2005). Crabs are transported in these totes to processing facilities. During extended holding periods, the crabs are placed in refrigerated storage or indirect contact with ice which slows their metabolism and bodily functions while extending their shelf-life.

Processing steps commonly implemented in the blue crab industry for fresh crab meat include: cooking, air cooling, refrigerating, hand picking, packing and boxing with ice for shipment (Senkel et al., 2005). Pasteurized crab meat goes through a similar process; however, it is packed hermetically with a strong seal, pasteurized and placed under refrigerated storage with an extended shelf-life. Crab can also be marketed as alive, fresh whole cooked, frozen whole-cooked, frozen sections or clusters and frozen “snap and eat” legs. To reduce significant amounts of bacteria, crabs which are sold in clusters are de-backed, de-gilled and eviscerated (Senkel et al., 2005).

In the U.S., the three major market forms of crab meat include fresh, pasteurized and frozen. After crabs are caught, they are further sorted by size, inspected for chemical contamination and mortality, and transported to small crab houses or processing plants where they are then steamed or boiled. Their meat is then extracted by hand which yields crab meat ranging from jumbo lump to small bits of leg meat. Generally, the claws and knuckles are separated first, then the back, gills and apron are



removed which provides easy access to carapace meat which can be extracted using picks. Meat from the claws and knuckles is accessed by crushing the shell using a wooden mallet. Rollers are used to squeeze out any meat remaining in the legs. Experienced blue crab workers can “pick” as much as one crab every thirty seconds (Senkel et al., 2005). Well-picked Dungeness or blue crab can yield up to 25 % meat by weight; however, yields of 10 to 20 % are more common with approximately 85 % of residual meat designated as crab shell by-product.

During the year 2000, it cost \$0.07/kg (\$61/ton) to landfill crab by-products in the state of Maine (Wentworth et al., 2002). This equates to over \$1.5 million per year for crab processors (Seymour et al., 2001; Wentworth et al., 2002). Most of the crab by-product designated as waste still contains a substantial quantity of residual meat in the legs and body. Prior research has explored the use of mechanical separation or ‘deboning’ to extract crab meat from pre-picked crab shells. Mechanical separation of minced meat from crab by-products yields approximately 15 % to 20 % meat from discarded crab shell by-product and reduces waste (Gates and Parker, 1992a; Gates and Parker, 1992b; Gates and Parker, 1993). In a Paoli style mechanical separator, crab shell discards are introduced through a large feed hopper and are then ground into fine pieces by a large rotating metal cylinder which contains microslits for the meat to pass through. The cylinder contains serrated pieces of metal along its external surface to facilitate grinding. Crab meat is finely ground into a mince and is forced through the microslits of the cylinder where it exits under the machine (Gillman, 2001). Shell fragments remain on the outside of the cylinder and exit through the end plate.

Other types of mechanical separators and other methods of meat extraction have been explored and include: shaking the meat out of the shell by vibration, using a vacuum, squeezing the meat out of the shell with rollers, removing the meat from the shell by centrifugal force, and crushing the shell with a hammer mill and allowing the meat to float to the top of a brine solution for extraction (Ward, 1990). More research is needed in these areas to develop a dependable high-quality crab meat product.

Most crab meat products are considered ready-to-eat and are generally at high risk for bacterial contamination due to post-cook handling (Senkel et al., 2005). *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus* are the three major pathogens of concern in fresh crab meat. Pasteurized crab meat is at risk from *Clostridium botulinum*, especially type E due to its psychrophilic nature. There are many opportunities for contamination due to the high amount of post-cook handling and the microbial status of the crabs' environment. Most of the bacteria present on the crab are located on the shell, viscera and gills. Crab meat is an excellent medium for bacterial growth due to its high water activity, moderate pH and low salinity. Although food processors are able to control these factors with acidification, the addition of salts and preservatives and other hurdle techniques; most seafood processors do not incorporate preservatives due to detrimental sensory effects. Pasteurization, refrigeration and oxygen removal are the three main hurdles used in the processing of fresh crab meat.

Although crab meat is at high risk for microbial contamination, only a few incidents of illness have been reported (Senkel et al., 2005). Researchers have suggested that few pathogens are able to compete with overwhelming spoilage bacteria because

crab meat is a good medium for bacterial growth (Suklim et al., 2008). Fresh crab meat generally has a refrigerated shelf-life of three to five days after purchase. Spoiled crab meat has a sticky texture and is yellowish in color. An ammonia odor may be present due to the degradation of protein and nitrogenous compounds from bacterial and enzymatic action.

### **Composition**

Understanding the composition of green crab is vital for subsequent processing and value-added production. Naczek et al. (2004) determined the total protein content, lipids, and carotenoids in green crab meat, on a dry weight basis, to be 80.6-83.5 %, 3.6-4.8 %, and 5.1-19.2 mg %, respectively. Skonberg and Perkins (2002) also studied the nutrient composition of green crab and found similar results. They concluded averages of 78.7 (moisture), 17.1 (protein), and 2.2 (total mineral content) g/100 g of the crab meat on a wet weight basis. Fat content was also analyzed in this study showing a significant difference between leg meat and both raw and steamed claw meat. Fat content of leg meat averaged 1.2 g/100 g, whereas fat content of claw meat averaged 0.6 g/100 g (Skonberg and Perkins, 2002). When these numbers are converted from a wet to a dry weight basis, there is a strong similarity in results between the two studies.

An interesting study performed by Cherif et al. (2008) showed differences in composition between green crab claw meat and the hepatopancreas. The hepatopancreas is the organ in the crab responsible for digestive functions that are performed by the pancreas, intestine and liver in mammals. Cherif et al. (2008) first

analyzed moisture, crude protein and fat content, showing results of approximately 79.9-81.2, 17.8-18.2 and 0.85-1.0 %, respectively, for green crab claw meat.

Hepatopancreas moisture, crude protein and fat contents were reported at 64.5-65.5, 13-14, and 21.8-22.7 %, respectively. The major omega-3 polyunsaturated fatty acids analyzed in this study included eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA). Omega-3 fatty acids were 23.42-25.21 % and 21.54-22.26 % of total fat in the hepatopancreas and claw meat, and 58-59 % and 59-61 % of all polyunsaturated fatty acids in the hepatopancreas and claw meat, respectively (Cherif et al., 2008). These values are lower than those reported by Naczek et al. (2004) for Nova Scotia green crab, but similar to those reported by Skonberg and Perkins (2002) for green crab caught in the Gulf of Maine. The observed difference in the EPA and DHA content in this study compared to that reported by Naczek et al. (2004) may be due to differences in the crab diet. EPA and DHA are essential polyunsaturated fatty acids that are used in the human body as important building blocks for retina and brain development. They are especially important during fetal development and infancy, and have been reported to maintain healthy cardiovascular function in adults.

Research has been conducted on the chemical composition and properties of crab shells which is important in research and development for total product utilization. Naczek et al. (2004) reported that crab shell discards contained 12.6-14.5 % of chitin, 2.6-3.11 % of total nitrogen, 0.37-0.65 % of total lipids, and 4.4-9.3 mg % of total carotenoids on a dry weight basis. Similar values for chitin were reported by Muzzarelli (1977) and Johnson and Peniston (1982) who determined that crab shell was comprised

of 13 % to 15 % of chitin, on a dry weight basis. In comparison, 40-50 % of total shrimp weight is designated as by-product, while 40 % of this by-product is chitin.

A study performed by Yen et al. (2008) investigated whether crab chitosan has good antioxidant activity, scavenging ability on hydroxyl radicals and chelating ability on ferrous ions. Results of this study were compared to commonly used antioxidants such as  $\alpha$ -tocopherol, BHA and ascorbic acid. These results indicate that crab chitosans contain good antioxidant activity, have the ability to scavenge hydroxyl radicals and are strong ferrous ion chelators (Yen et al., 2008). This study supports the potential use of crab shell by-product within value-added food products or pharmaceutical supplements.

Soft shell crab is becoming increasingly popular around the world and is usually sold at higher values compared to hard shell crab. In a comparative study of hard shell and soft shell mud crabs, Benjakul and Sutthipan (2009) reported a significant difference in calcium content, as well as other minerals, between pre-molt and post-molt lump and claw meat. For example they found means of 699.25 mg/kg of calcium in hard shell lump muscle and only 240.57 mg/kg of calcium in soft shell lump muscle of mud crab. These results agreed well with results obtained from Scott-fordsmand and Depledge (1997) who also showed lower whole body calcium concentrations in freshly molted green crabs (*Carcinus maenas*) (Benjakul and Sutthipan, 2009). Studies have also shown that there is greater moisture and less protein content in soft shell compared to hard shell crabs due to physiological changes in the crab during molting.

## **Maintaining Crab Meat Quality**

Converting whole green crab into mince by mechanical separation is inherently a size reduction unit operation and can result in the incorporation of shell fragments, or 'shell-flour,' within the meat which causes textural issues. An increase in surface area promotes oxidative effects and increases rates of microbiological and enzymatic activity (Nawar, 1996). When crab cells are disrupted through mechanical processing, enzymes and spoilage organisms readily combine with suitable substrates. This results in the accelerated deterioration of crab mince sensory attributes; such as odor, color and flavor, compared with lump crab meat. Preservative measures must be taken to slow the rates of oxidative, microbiological and enzymatic actions. Developing methods to prevent or reduce chemical and microbiological deterioration is important in the development of value-added products from crab mince meat (Gillman and Skonberg, 2001).

Oxidation is a potential problem in any food containing significant amounts of fat. Although green crab mince generally contains only about one percent total lipids, oxidation may take place during longer periods of frozen storage. Metals scraped from the machine during mechanical separation can increase oxidative effects (Gillman and Skonberg, 2001). In addition, virtually all minced products have increased surface area which promotes oxidative actions. Oxidation causes the deterioration of lipids to hydroperoxides to form a wide variety of hydroxy compounds, carbonyl compounds and short chain fatty acids. The deterioration of lipids results in very detectable off odors and flavors (Nielsen and Jacobsen, 2009). Butylated hydroxyanisole (BHA), butylated

hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), propyl gallate (PG), ascorbic acid,  $\alpha$ -tocopherol, ascorbate, and erythorbic acid are additives that have been utilized in seafood as antioxidant agents.

Low dose gamma radiation has proven effective in reducing spoilage and pathogenic microorganisms in crab meat products (Chen et al. 1996). Crab mince is a suitable product for irradiation because it generally contains small amounts of fat. Products with a high fat content encounter substantial oxidative effects during irradiation which results in unacceptable changes to odor, flavor and overall consumer acceptability. In addition, irradiation is considered a non-thermal processing technique which helps preserve nutritional and sensory qualities in low-fat products.

Chen et al. (1996) investigated the sensory and microbial qualities of irradiated crab meat products during fourteen days of iced storage. Results showed a three day shelf-life extension in the irradiated crab meat products with off-odors and off-flavors developing more rapidly in the untreated samples. Overall acceptability scores were higher for irradiated crab samples compared to control samples throughout 14-days of iced storage (Chen et al., 1996).

High hydrostatic pressure processing (HPP) is a relatively new and expensive technology used in the industry to greatly reduce microbial activity without treating the product with high temperatures. High pressure forces the collapse of intracellular vacuoles and damages cell walls of microbes (Fellows, 2000). This process is effective in retaining sensory characteristics and nutritive quality of seafood products relative to high temperature processing. One major limitation is that enzymes generally remain

functional unless treated with very high pressures (>1000 MPa). Other non-thermal, and relatively new technologies used to reduce microbial activity include the use of ultraviolet light, pulsed electric fields, pulsed light and ultrasound waves.

Suklim et al. (2008) investigated the effects of high hydrostatic pressure processing on the germination and inactivation of *Bacillus cereus* spores in fresh blue crab meat (*Callinectes sapidus*). Bacterial spores are resistant to environmental stresses including: radiation, heat, drying and chemical agents (Suklim et al., 2008). They can survive for long periods of time and under the proper environmental conditions and can germinate to produce vegetative microbial growth, which can produce toxins in food. Suklim et al. (2008) showed that pressure-induced germination of *B. cereus* spores and subsequent pressure processing combined with moderate thermal treatment (550 MPa, 40°C, 15 min) effectively reduced the risk of *B. cereus* foodborne illness. Although the total destruction of *B. cereus* spores was not achieved, natural blue crab microflora was competitive enough to limit *B. cereus* counts below pathogenic levels even after all of the crab meat was subjected to high pressure processing.

Currently, the primary method of seafood inspection is performed by sensory evaluation (Sarnoski, 2007). Well trained seafood inspectors are able to use all five senses to qualitatively determine the quality of crab meat products. Using their senses, inspectors can efficiently check for: visual discolorations, malformations, off-odors and flavors, off-colors, textural inconsistencies and can even listen for proper 'squishiness' or 'pop' during cooking. Until technology is further developed to efficiently assess multiple quality attributes of seafood products to the perceptive levels of human



senses, human sensory evaluation will be the primary method of determining seafood quality. The easiest approach to assessing food quality would be a quick and cheap chemical or instrumental test that could measure several quality attributes simultaneously (Sarnoski, 2007). In addition, quantitative data from analytical testing is far more consistent and less subjective compared to sensory evaluation between inspectors due to the use of established analytical protocols and the reduction of sensory bias or variability.

Many studies have been performed in which quantitative technological data are overlaid with sensory data to show correlations of specific quality attributes. One such study performed by Sarnoski (2007) was to determine key indicators of spoilage in crab meat using an electronic nose, volatile amine measurements and gas chromatography-mass spectrometry (GC-MS). An electronic nose is an instrument comprised of an array of chemical sensors combined with a pattern-recognition system used to detect complex odors. Total volatile bases (TVB) such as trimethylamine, dimethylamine, biogenic amines and ammonia were tested. The GC-MS analyses were used to determine which chemicals were present in high quality, mid-quality, low quality and spoiled crab meat, which was used to subsequently determine if they can be used as dependable quality indicators for blue crab meat. Results indicated that improvements in electronic nose technology are needed before it can be used as a reliable quality indicator for crab meat. GC-MS found ammonia, trimethylamine and indole to correlate well with the spoilage of blue crab meat. However, trimethylamine is connected with microbial spoilage, and microbial growth increases exponentially whereas,

trimethylamine increases linearly. Indole agreed well with sensory results suggesting indole as an indicator for detecting spoiled samples. Ammonia amount did not correlate with sensory and aerobic plate counts as well as indole, but did reflect an exponential curve similar to microbial spoilage. Ammonia may be a better chemical indicator of microbial growth than trimethylamine in seafood (Sarnoski, 2007).

A variety of natural and synthetic additives have been used to influence the consumer acceptability, shelf-life and yield of cooked crab meat (Gillman, 2001). To regulate the acidity of canned crab meat; citric acid, orthophosphoric acid or disodium diphosphate are commonly used. Calcium disodium ethylenediaminetetraacetic acid (EDTA) is sometimes used as a metal chelator or sequestrant, and monosodium glutamate (MSG) is used for flavor enhancement. Cryoprotectants are used in the surimi industry to stabilize crab and fish mince which increase their functionality and frozen stability. Surimi cryoprotectants usually include a combination of sugars, sorbitol and polyphosphates.

Crapo and Crawford (1991) investigated the influence of polyphosphate soaks and cooking procedures on yield and quality of Dungeness crab (*Cancer magister*) meat. Raw crab sections were soaked in polyphosphate solutions and cooked by boiling or steaming for varying times. The most favorable meat yield, quality and frozen storage stability was reached by soaking raw sections for more than 60, but less than 120 min in 10 % polyphosphate (2-4°C) and then cooking in steam for approximately 8 minutes (Crapo and Crawford, 1991). Phosphate soaks are traditionally used to increase

moisture retention, chelate pro-oxidant metals and improve texture in the seafood and meat industries.

A study performed by Gillman and Skonberg (2001) evaluated the effects of sodium lactate, lactic acid, rosemary, and diacetyl on the chemical and microbial quality of refrigerated crab mince. Sodium lactate acts primarily on gram-negative bacteria by lowering pH and making the surrounding environment unsuitable for bacterial growth. Lactic acid works similarly by influencing cell structure and pH of primarily gram-negative bacteria. Rosemary is generally used as a natural antioxidant, but has also shown antimicrobial effects in food products. Diacetyl is a common additive in the dairy industry due to its buttery flavor profile and antimicrobial effectiveness against molds and gram-negative bacteria. The addition of lactic acid had the most noteworthy effect in improving the shelf-life of mechanically separated crab mince; however, additions of sodium lactate, lactic acid, rosemary, and diacetyl were also effective (Gillman, 2001). The lactic acid addition reduced the microbial load of crab mince by half of a log although the reduction was not statistically significant. Gillman (2001) suggested that combinations of additives may be most effective in retaining the quality of refrigerated crab mince. For example, lactic acid may be able to disrupt microbial cell membranes, permitting anti-microbial compounds found in rosemary to enter which could inhibit microbial growth. The addition of 5 % rosemary plus 2 % lactic acid resulted in significantly ( $p < 0.01$ ) lower microbial counts compared to the control treatment.

Other antimicrobial additives typically used in the seafood industry include: acetic acid, citric acid, potassium lactate, nitrates, phosphates, sorbitol, and salts. Gates

and Parker (1993) investigated the use of citric acid phosphate buffer in blue crab minced meat in aluminum cans under frozen and refrigerated storage. Results showed crab mince meat treated with the citric acid phosphate buffer before pasteurization in aluminum cans had a better color than unbuffered meat throughout 11 months of frozen storage (Gates and Parker, 1993). Citric acid phosphate buffers reduced enzyme function and subsequently limited enzymatic color change in crab meat.

Elevated microbial values and dark color are limitations in the marketability of fresh crab mince. Packaging is an important step in maintaining the quality of a crab meat product. Oxygen barrier packaging should be used for crab mince meat if frozen storage times exceed 6 months (Gates and Parker, 1993). Often, pasteurized crab meat is stored in aluminum cans; however, the advent of thermally stable polyethylene packaging allows crab products to be sold in laminated pouches. Fresh, unpasteurized crab meat is often sold in plastic quarter or half-gallon containers in the consumer market with little regard to oxygen permeability.

### **Value-Added Crab Mince Products**

Crab by-product is primarily used in the production of aquaculture and agricultural feeds. The feed industry produces inexpensive, high quality feeds using amino acid hydrolysates from high protein waste products, such as crab by-product, in combination with grains. In light of decreasing fishery stocks and increasing population numbers worldwide, this underutilized high quality source of protein may be better suited for developing value-added food products. Currently, there are few products

made with crab mince available in the consumer market. Minced meat crab by-products can be used as extenders in seafood stuffings, chowders and soups (Gates and Parker, 1992b). The cost for minced crab meat is much lower than the cost of claw meat, but the cost of minced meat is approximately 20 times the value of crab meal sold to animal and fish feed companies (Gates and Parker, 1992b).

Surimi is a washed, gelled and cooked fish mince that is used to mimic fish or crab products. Surimi represents a highly successful utilization of fish mince in the production of value-added food products and may be a potential product for green crab mince utilization. Surimi has been proven in the consumer market with products such as: surimi nuggets, sticks, patties and sausages. Surimi is a very versatile product because it can be made from different combinations of fish mince, and a variety of flavors and colorants can be added to produce a variety of different surimi products. It has the ability to form many different shapes and can be sold at relatively high prices even though the price can vary widely depending on color and quality. Baxter (2007) demonstrated the gelation properties of previously cooked Jonah crab mince indicating its ability to retain functionality. Jonah crab mince meat was shown to form gels which may facilitate the development of value-added crab products.

A major problem that may occur during the development of a value-added crab mince product is related to texture. The product may have a “gritty” mouth feel due to the small shell particles migrating into the mince during mechanical separation. Gillman (2001) investigated the use of mince from crab processing by-product in the development of pasta with accompanied sensory and quality analyses. Comments from

sensory panelists indicated that the pasta had a slight seafood flavor and a gritty texture because of residual shell particulates in the pasta. Although textural issues were noted, successful crab mince pasta was developed.

It can be certain that if the European green crab enters the consumer food market, exploitation of its entire biomass, such as potential uses and extraction of chitin, will be investigated and developed if feasible. Naczek et al. (2004) determined green crab shells to be comprised of approximately 14 % chitin on a dry weight basis. It is one of the most important biopolymers, and is comparable to cellulose and the protein keratin in both structure and function. Chitin is found naturally in crustaceans, mollusks, insects and fungi and about 100 billion tons is extracted annually (Tharanathan and Kittur, 2003; Xu et al., 2008). A substantial quantity of chitin is extracted from the by-products of marine food production, such as shrimp and crab shells or krill (Xu et al., 2008). Chitosan is derived from chitin by partial N-deacetylation. Research has explored the use of chitin and chitosan for controlled drug release, anticancer applications, improved wound healing, food and nutrition, artificial skin and cosmetics (Felse and Panda, 1999; Dutta et al., 2002; Dolphen et al., 2007; Xu et al., 2008). In the food industry, chitin and its derivatives have applications as emulsifying, antimicrobial, thickening and stabilizing agents. Chitosan has also been an effective food coating for reducing moisture loss and inhibiting microbial growth (Li et al., 1997).

Crab shell has been used in the past as part of total product utilization in addition to the production of chitin and chitosan. Carotenoid pigments, flavorants and enzymes have been extracted from crab by-products (Shahidi, 1995). Some areas of the

food industry use the volatile flavor compounds from the shell by-product to make bases, stocks, and soups to provide a genuine crab flavor (Meyers, 1994). Aqueous extracts recover shellfish flavorants from shell discards or cook water, while carotenoids are extracted into oil or by using enzyme-assisted technology (Shahidi, 1995). The oil may be reused as part of an enrichment process and as an additive in the production of aquaculture feeds, especially for salmonid fish species.

### **Meat Restructuring**

Meat restructuring technology is useful in meat or fish processing operations where there is a lot of skeletal muscle by-product and enables the production of value-added products from low quality trimmings. Restructured meat products are generally formed to mimic the color, flavor, textural properties and often the shape of fresh muscle; as seen in the production of molded fish filets from pacific whiting or chicken patties from poultry by-products. Yada (2000) suggested the use of protein isolates to produce films and coatings for the preservation of food products.

The production of restructured meat products first involves a maceration step where meat is ground into a paste and various additives can be incorporated, such as starch, isolated soy protein, phosphate, egg white, beef plasma protein, transglutaminase, sodium chloride, humectants, sorbitol, sugar or seasonings. Additions of sodium chloride, phosphates or other water-competitive components dissociate proteins which increases protein solubility (Zayas, 1997). At this point, gel networks are formed through a cold-set or thermal-set process depending on the additives used and

the desired end product. Thermal setting of gels involves a cooking process which causes cross-linking of amino acid residues, thus forming a tight protein network and entrapping water. For products to be sold as raw or chilled, a cold-set process is required. Additives such as transglutaminase act by cross-linking amino acid residues without the need for high temperatures. Restructured meat products which have been cold-set are generally not as strong as thermally set products. Hydrogen bonding, disulfide bonding, and hydrophobic and electrostatic interactions are interactions which stabilize protein gels.

The addition of sodium chloride is especially important in enhancing the binding ability in protein gels by extracting salt-soluble proteins, such as myofibrillar proteins, which act as binding agents among water and meat particles. However, diets high in sodium chloride have been associated with cardiovascular disease and food manufactures are now substituting for salt in their products. The reduction of sodium chloride in restructured meat products adversely affects water-holding capacity and gel strength, which subsequently results in degraded textural properties and overall cook yield (Zayas, 1997). Additives such as: microbial transglutaminase, isolated whey protein, dried egg white, sodium caseinate, isolated soy protein and other nonmeat proteins have been shown to overcome deficiencies in water-holding capacity and textural properties in low sodium restructured meat products.

Often in the production of restructured meat products, endogenous proteolytic enzymes are present which cause the degradation of actomyosin and reduce overall gel strength. This issue is further complicated by the fact that these enzymes are highly



active at those temperatures required for the setting of restructured meat proteins. To overcome this problem, processors incorporate different protease inhibitors during the maceration process to increase the overall effectiveness of restructuring protein additives.

Functional properties responsible for the texture and water-holding capacity in cooked restructured meat products include: solubility (protein-extraction), emulsifying capacity, water binding ability and gelation (Yada, 2000). Physicochemical and biochemical properties of muscle proteins change due to denaturation during cooking which cause a loss of functionality. Protein denaturation involves a significant change in a protein's native structure or molecular shape. Heat or a significant change in pH are the two most common reasons for protein denaturation in the food industry, and most often result in the production of desirable food products. However, denaturation causes a loss of solubility and water-holding capacity because of the exposure of sulfhydryl groups and hydrophobic amino acid residues during unfolding. Denaturation and subsequent loss of protein functionality can be averted with the use of transglutaminases.

Transglutaminases are enzymes which catalyze the creation of a cross-link between a free amine group and the  $\gamma$ -carboxamide group of protein-bound or peptide-bound glutamine (Zhu and Tramper, 2008). They produce non-disulfide covalent bonds between lysine and glutamine residues. These enzymes are useful in promoting textural improvement, meat restructuring, gel formation and an increase in overall nutritional value in food processing. Min and Green (2008) studied the effects of transglutaminase

additions ranging from 0.05-0.7 % by weight in channel catfish patties. The addition of microbial transglutaminase increased binding strength, hardness, cohesiveness, chewiness, and springiness, but decreased cooking yield of the patties (Min and Green, 2008). In addition, transglutaminases have found a niche in meat processing operations which produce low-fat or fat-free products, and bonds formed by transglutaminase have high resistance to enzymes which cause proteolytic degradation.

Transglutaminases have been found in a variety of plants, animals and microorganisms. The majority of transglutaminases currently used in the food industry are from microbial origins: specifically *Streptovorticillium mobaraense*, *Streptomyces mobaraense*, *Bacillus subtilis*, and *Physarum polycephalum* species. The advent of using microorganisms for transglutaminase mass production has resulted in lower costs of production than from the purification of transglutaminase from animal tissues. In addition, microbial transglutaminases are calcium-independent, unlike their animal counterparts.

Isolated soy protein is another additive used to increase the functionality of restructured meat products. It is made from defatted soy flour and is highly purified. One of its major roles in the food industry is to improve the texture of meat products. Soy protein preparations can increase the functionality of food systems by altering water absorption and binding, solubility, gelation, viscosity, cohesion-adhesion, emulsification, elasticity, flavor-binding, fat adsorption, foaming and color control (Yada, 2000). These properties are also important in facilitating processing.

Isolated soy protein has the advantage of being cheap and easily spun into fibers which can form restructured foods simulating fibrous meats. Although most texturized soy products are made from soy flours, isolated soy protein can be compressed into a hot, flowing plastic mass and extruded, forming a structure similar to meat fibers. The extruded soy fibers can then be spun, colored, flavored and shaped to further simulate meat products. Although soy protein has the potential to be used in a wide variety of food products, it has been associated with grassy, beany and chalky off-flavors which are caused by isoflavones and enzymatic breakdown products and have a negative influence on consumer acceptability (Childs et al., 2007). Manufacturers of soy proteins adopt the best methodologies to reduce these off-flavors. In addition, cultivators have been growing genetically modified soybeans which have reduced allergenic potential and can reduce enzyme production, which allows soy proteins to be used in a broader spectrum of food products.

Egg whites are another useful additive in the production of restructured meat products. Studies have shown egg whites to significantly increase the gel strength and overall texture profile of restructured meat. Baxter (2007) showed that additions of dried egg whites in a restructured Jonah crab product significantly increased hardness, springiness, cohesiveness and gumminess compared to the control. When using dried egg whites, it is important to thermally set the protein. Cooking initiates denaturation which results in the exposure of hydrophobic and sulfhydryl groups. Gel networks are then formed via disulfide bonds and hydrophobic interactions. Egg whites contain

albumin which is the most bioavailable protein. However, they have shown to make end products glossier and whiter, which can result in lower consumer acceptability.

## **Conclusions**

The European green crab (*Carcinus maenas*) has proven to be an invasive species worldwide, preying on bivalves and mollusks, and competing with other crustaceans. Green crab's high tolerance to environmental extremes and its strong reproductive output are factors that contribute to its invasive nature. Despite green crab prevalence, there is little to no commercial exploitation due to its extremely small size. Green crab contains high quality protein and other beneficial nutrients. The development of value-added green crab mince products may help establish a commercially viable green crab fishery which may help to control this nuisance species.

## **Objectives**

The overall objective of this research was to help develop a commercial use of green crabs by examining mechanical separation techniques; developing a consumer acceptable mince meat product and exploring the functionality of green crab mince meat containing restructuring additives. This research was completed in a series of three studies.

The specific objectives of these studies were:

1) To determine how boil versus steam thermal processing, large (>55 mm carapace width) versus small (<55 mm carapace width) crab size, and 0.000 versus 0.180 breaker bar setting on the mechanical separator affects green crab mince meat yield and quality.

2) To develop a consumer acceptable empanada containing green crab mince and to determine how consumer acceptability of empanadas are affected with additions of 30, 50 and 70 % green crab mince by filling weight.

3) To determine how additions of 1, 2 and 4 % transglutaminase, as well as additions of 5 % isolated soy protein, 5 % dried egg white and their combinations with 2 % transglutaminase affects the textural profile, color ( $L^*a^*b^*$ ), cook yield, water-holding capacity and proximate composition of fully cooked green crab mince patties.

## CHAPTER 2. MECHANICAL SEPARATION OF EUROPEAN GREEN CRAB (CARCINUS MAENAS) AND THE EFFECTS ON MINCE MEAT YIELD AND QUALITY

### Objectives

The objectives of this study were to determine the effects of thermal processing, crab size and mechanical separator breaker bar setting on green crab mince meat yield and quality.

### Materials and Methods

#### Experimental Design

Three variables were tested in this study: boil versus steam thermal processing, large (>55 mm carapace width) versus small (<55 mm carapace width) crab size, and 0.000 versus 0.180 breaker bar setting on the mechanical separator. The combinations of treatment variables can be seen in Table 1. Each treatment was processed in duplicate.

Table 1 Treatment Codes and Variables Used in the Experimental Design

Treatment	Code	Cook Method	Crab Size	Breaker Bar Setting
1	BL00	Boil	Large	0.000
2	BL18	Boil	Large	0.180
3	BS00	Boil	Small	0.000
4	SL00	Steam	Large	0.000
5	SL18	Steam	Large	0.180
6	SS00	Steam	Small	0.000

### Mechanical Separation

The mechanical separator used in this study was a Paoli One-Step mechanical separator Model 22-849 (Rockford, IL). It required intense cleaning between treatments to ensure the removal of shell material, which can decrease yield. The cylinder is the part of the mechanical separator which facilitates the grinding action. The cylinder contains microslits which are easily blocked with food particles if not cleaned often. The breaker bar allows for adjustment in the degree of closeness of the grinding action with the cylinder. A setting of 0.000 represents as tight as the grinding action will allow, and a setting of 0.180 opens the grinding cavity as wide as possible.

To begin the study, the cylinder was heated in a 15 % phosphoric acid (Fisher Scientific Inc.; Hampton, NH) bath to 90° C for eight hours. This removed any particulate material trapped in the microslits of the cylinder. The microslits of the cylinder were further cleaned with the use of a feeler gauge blade (0.006"). The machine was then sanitized using dish detergent, lubricated with vegetable oil and re-assembled for processing with the end cap set at ¼ open. These cleaning steps occurred between each of the twelve batches and at the conclusion of the study.

Two hundred and forty pounds of green crab was freshly caught off the coast of Swan's Island, ME, delivered to the University of Maine Dr. Matthew Highlands Pilot Plant, and separated into twelve, twenty pound batches (6 treatments in duplicate). Upon separation into batches, green crabs were individually inspected for foreign material and mortality. Dead green crabs were discarded. Ten crabs from each batch were measured for carapace width to the nearest tenth of a millimeter using a

Traceable® digital caliper Model 14-648-17 (Friendswood, TX) to determine average crab size and minimize variability among treatments. Green crabs were separated based on size into two treatment groups: >55 mm carapace width and <55 mm carapace width. Total live weight was recorded in grams for each batch, with approximately twenty pounds per treatment. Green crabs were labeled according to their batch and held under refrigeration temperature until they were ready to be processed.

For each batch of crabs to receive a boil treatment, 50 liters of potable tap water were brought to 100° C in the Dr. Matthew Highlands Pilot Plant's steam jacketed kettle. The temperature of the water was monitored using a Type K Fisher Scientific Thermocouple (Pittsburgh, PA). The crabs were then dumped into a large metal colander and fully submerged into the boiling water. The temperature of the water was allowed to return to 100° C (~1 min) and the crabs were cooked for an additional 7 minutes. Once the boiling was finished, the crabs were removed from the water and immediately checked for an internal temperature of at least 74° C (165° F) by inserting a thermocouple wire through the eye socket of the three visibly largest crabs. Once the internal temperature was verified, crabs were submerged into a large ice water bath, stirred and allowed to cool below 21° C. Internal temperatures were taken and recorded using the same method as previously mentioned. Once the crabs were cool, they were placed onto a large wire rack over the sink. The crabs were spread evenly and sprayed with cold water to melt any residual ice. The wire rack was shaken, and the crabs were air dried for approximately 5 minutes. The crabs were then placed into a pre-tared plastic container and re-weighed. Post-cook weight was recorded in grams.



For each batch of crabs to receive a steam treatment, the basin of the Cleveland SteamCub Model 1SCEMSC (Cleveland Range Inc.; Cleveland, OH) steamer located in the Department of Food Science and Human Nutrition's Commercial Kitchen was filled with 2 liters of potable water. The steamer was turned on and placed on 'hold', which brings the water close to a temperature of 100° C. Live crabs were removed from the refrigerator, and placed into five perforated stainless steel half-hotel pans. The hotel pans were then inserted into the steamer and the temperature was allowed to return to 100° C (~3-4 minutes). The steamer was then set to 'steam', which boils the water in the basin of the steamer, and the crabs were cooked for an additional 7 minutes. After 7 minutes, the crabs were removed from the steamer and immediately checked for an internal temperature of at least 74° C (165° F) using the same method as outlined for boiling. Once internal temperature was verified, crabs were submerged into an ice-water bath, stirred and allowed to cool below 21° C. These steps were repeated since 10 pounds of each 20 pound batch of crabs could be evenly spread onto the perforated half-hotel pans at one time. Once the crabs were cool, they were placed onto a large wire rack over the sink. The crabs were spread evenly and sprayed with cold water to melt any residual ice. The wire rack was shaken, and the crabs were air dried for approximately 5 minutes. The crabs were then placed into a pre-tared plastic container and re-weighed. Post-cook weight was recorded in grams.

For each batch of crabs processed, regardless of whether they were steamed or boiled, the mechanical separator end cap was opened  $\frac{1}{4}$ , and the breaker bar was adjusted according to each treatment. Two pre-weighed, plastic collection containers

were placed under the mechanical separator. One was used to catch the crab mince and the other was used to catch shell discards. The machine was turned on and the cooked crabs were fed into the feed hopper until all of the crabs were processed. The machine was turned off and unplugged, and using a clean spatula, residual mince meat which had not fallen into the collection container was scraped down. The mince and shell discards were weighed in grams. There was residual water which was left in the plastic container used to weigh the cooked crabs. This water was weighed and subtracted from the post-cook weight of the crabs. The shell discards were thrown away and the crab mince was mixed well using a sanitized spatula. Approximately 20 grams of crab mince were placed into two Whirl-Pak stomacher bags (Nasco; Fort Atkinson, WI) each, and subsequently used for microbial analysis. Approximately 454 grams of crab mince were placed into a plastic Ziploc® bag, labeled and subsequently used for moisture, pH, TVBN and color analyses. The remainder of the crab mince was placed into a plastic Ziploc® bag, labeled, frozen and subsequently used for crude protein, crude lipid and ash analyses. The mechanical separator was cleaned between each batch according to the methods already outlined.

### Yield

Green crab mince meat yield was calculated two ways: pre-cook yield and post-cook yield. Pre-cook yield was determined by dividing final crab mince meat weight by pre-cook weight and multiplying by 100. Post-cook yield was determined by dividing final crab mince meat weight by post-cook weight and multiplying by 100.

### Moisture Determination

Each batch was analyzed for total moisture in triplicate. Aluminum drying pans were labeled according to treatment and replication number. Pans were weighed on an analytical balance Model HR200 (A&D Co. Ltd.; Tokyo, Japan) to the nearest 0.001 g, and pan weight was recorded. Samples were homogenized using a Black and Decker® Handy Chopper Plus (Household Products Inc.; Shelton, CT). The pans were tared and approximately 5 grams of homogenized sample was spread evenly on the pan. The weight of each sample was recorded to the nearest 0.001 g. Each sample was placed in a 100° C Fisher Isotemp draft oven Model 350 (Fisher Scientific; Pittsburgh, PA) for at least 6 hours but no greater than 10 hours. The pans were removed from the oven and allowed to cool. They were then re-weighed to the nearest 0.001 g and recorded. Total moisture content was analyzed based on AOAC method # 950.46 (AOAC, 2005).

Moisture was determined using the following calculation:

$$\frac{(\text{pan wt.} + \text{sample wt.}) - (\text{pan} + \text{dry sample wt.})}{\text{sample wt.}} \times 100$$

### Crude Lipid Determination

Crude lipid content was determined in triplicate for each batch by using acid hydrolysis. Flat bottom flasks (~150 mL) were labeled and dried for approximately 10 minutes in a 100° C Fisher Isotemp draft oven Model 350 (Fisher Scientific; Pittsburgh, PA). They were removed from the oven, allowed to cool in a desiccator, and their weight was recorded on an analytical balance Model HR200 (A&D Co. Ltd.; Tokyo, Japan) to the nearest 0.001 g. Approximately 8 grams of crab sample were placed into a screw top

glass bottle and sample weight was recorded to the nearest 0.001 g. Ten mL of 8.1 N hydrochloric acid (Fisher Scientific Inc.; Hampton, NH) were added to the bottles. The bottles were placed in an 85-90° C water bath for 90 minutes and the samples were shaken by hand at frequent intervals. They were then taken out of the water bath and allowed to chill on ice until cool to the touch. Seven mL of ethyl alcohol (Fisher Scientific Inc.; Hampton, NH) were added to each bottle and shaken by hand for approximately fifteen seconds. Twenty-five mL of ethyl ether (Fisher Scientific Inc.; Hampton, NH) were then added to each bottle and shaken vigorously by hand for an additional one minute. Twenty-five mL of petroleum ether (Fisher Scientific Inc.; Hampton, NH) were then added to each bottle and shaken for another minute. The samples were allowed to stand until a two phase separation occurred. The top phase was clear and contained the fat and ethers while the bottom phase contained everything else. The upper clear liquid was then transferred using a glass pipette into the pre-labeled and pre-weighed flat bottom flask. The addition of ethyl ether and petroleum ether, and lipid extraction process, was repeated two more times with 15 mL of each. The upper clear liquid was transferred to the same flat bottom flasks for the last two extractions. The flat bottom flasks were then left under a fume hood overnight to allow the ether to evaporate leaving only the crude lipid. The flat bottom flasks were dried in a 100° C Fisher Isotemp draft oven Model 350 (Fisher Scientific; Pittsburgh, PA) for 10 minutes. The flasks were then allowed to cool in a desiccator and the final weight was recorded to the nearest 0.001 g. Total crude lipid content was analyzed based on AOAC method # 922.96 (AOAC,

2005). Crude lipid content was determined on a wet weight basis using the following calculation:

$$\frac{((\text{flask} + \text{lipid wt.}) - \text{flask wt.}) \times 100}{\text{sample wt.}}$$

#### Ash Determination

Crab mince samples were dried for 6-10 hours in a 100° C Fisher Isotemp draft oven Model 350 (Fisher Scientific; Pittsburgh, PA) and allowed to cool. Dried crab sample was crushed into a fine powder using a mortar and pestle. Scintillation vials were labeled using a diamond pen and weighed to the nearest 0.001 g. The vials were tared and approximately 1 gram of sample was added to each scintillation vial, and the final weight was recorded. The samples were placed in a 550° C Thermolyne Model F-A1730 muffle oven (Dubque, IA) for 6 hours. The samples were allowed to cool and re-weighed. Samples were analyzed in triplicate and total ash content was analyzed based on AOAC method # 938.08 (AOAC, 2005). Percent ash was determined on a dry weight basis (dwb) using the following calculation:

$$\frac{(\text{vial wt.} + \text{ash wt.}) - \text{vial wt.} \times 100}{\text{dry sample wt.}}$$

Percent ash was determined on a wet weight basis by using the following calculation:

$$\frac{(100 - \% \text{ average moisture content}) \times \% \text{ ash (dwb)}}{100}$$

### Crude Protein Determination

Nitrogen content of ~100 mg dried crab mince samples was analyzed using the Elementar Rapid N III combustion nitrogen analyzer (Elementar Americas Inc.; Mount Laurel, NJ). Crude protein was determined by multiplying % nitrogen by a 6.25 conversion factor. Samples were analyzed in duplicate.

### Total Volatile Base Nitrogen (TVBN)

Twenty-five grams of a crab mince sample and fifty mL of a 7.5 % trichloroacetic acid (TCA) (Sigma-Aldrich®; St. Louis, MO) solution were homogenized in a Microblender 700 Model 33BL79 (Dynamics Corp. of America; New Hartford, CT) for 30 seconds. The homogenized crab mince and TCA solutions were placed in centrifuge tubes (VWR; Boston, MA) and centrifuged at 1500 x g for 20 minutes at room temperature using a Beckman Coulter Avanti JE centrifuge Model JSE05B15 (Brea, CA). After centrifugation, 15 mL of supernatant were transferred using a glass pipette into the inlet cap of a micro-Kjeldahl distillation unit (Labconco Corporation; Kansas City, MO). The addition stopcock was turned which emptied the sample into the heating basin. Four mL of 10 % sodium hydroxide (Fisher Scientific Inc.; Hampton, NH) solution was transferred to the inlet cap and the stopcock was turned releasing the sodium hydroxide into the heating basin. The sides of the inlet cap were rinsed with distilled water. Total volatile base nitrogen, which volatilized from the crab mince sample during distillation, was captured in 15 mL of a 4 % boric acid (Fisher Scientific Inc.; Hampton, NH) solution containing 8 drops of methyl red-methylene blue indicator (Fisher Scientific Inc.; Hampton, NH)

solution (two parts 0.2 % alc methyl red solution with one part 0.2 % alc methylene blue solution). Samples which contained volatile base nitrogen caused a color change in the boric acid/indicator dye solution from purple to light green. The sample was then titrated with a 0.1 N hydrochloric acid (Fisher Scientific Inc.; Hampton, NH) solution until the color of the solution returned to the original purple color, which was compared against a blank. The amount of hydrochloric acid used was recorded. Samples were analyzed in triplicate and compared to 0.5, 1 and 2 mL of a total volatile base nitrogen standard solution (1800 mg ammonium sulfate (Bio-Rad; Hercules, CA) and 300 mg trimethylamine hydrochloride (Sigma-Aldrich®; St. Louis, MO) dissolved in 100 mL 7.5 % TCA). Total volatile base nitrogen was determined as milligrams of nitrogen per 100 grams using the following calculation:

$$(\text{mL HCl for sample} - \text{mL HCl for blank}) \times \text{normality of HCl} \times 14.007 \times (70 \text{ mL}/15 \text{ mL}) \times (100 \text{ g}/25 \text{ g}).$$

#### Aerobic Plate Counts (APCs)

Total aerobic plate counts were enumerated on petrifilm (3M Co.; St. Paul, MN). Nine parts 0.1 % bactopectone was added to the Whirl-Pak stomacher bags (Nasco; Fort Atkinson, WI) containing one part crab sample. Samples were then homogenized for 2 minutes in a Model 400 stomacher bag mixer (Tekmar Co.; Cincinnati, OH). Serial dilutions were performed in test tubes using 0.1 % bactopectone (DIFCO Laboratories; Detroit, MI) and ranged from  $10^2$  to  $10^7$  logs. Petrifilm was labeled according to batch, dilution factor and replication and placed on a clean level surface. One milliliter of sample was pipetted from each dilution to its corresponding petrifilm and a spreader

was used to distribute the sample over a circular area. The petrifilm plates were then incubated for forty-eight hours in a Fischer Scientific (Hampton, NH) incubator at 35° C. After incubation, bacterial colonies were counted on films that had a minimum of 25 and a maximum of 250 colonies. Final results were expressed as colony forming units per gram of sample. Each batch was analyzed in duplicate.

#### Color (L\*a\*b\*)

Samples of green crab mince were subjected to colorimetric analysis using a LabScan XE Hunter Lab colorimeter (Hunter Associates Laboratory; Reston, VA) to determine Hunter Lab L\*a\*b\* values. Samples were filled into a 2.5 inch clear glass sample cup with a black ring and disk, and placed on a pre-calibrated 2.5 inch sample port. L\*a\*b\* values were recorded using the computer software and each sample was turned 1/3 of a turn and read twice more. Analysis was completed in triplicate resulting in a total of six measurements per treatment.

#### pH

A 50:50 crab mince sample and distilled water mixture was homogenized in a Microblender 700 Model 33BL79 (Dynamics Corp. of America; New Hartford, CT) for 30 seconds. The sample was transferred to a small beaker and the pH was recorded in triplicate using the same homogenate with a Mettler-Toledo InLab Expert Pro pH meter (Mettler-Toledo Inc.; Columbus, OH).



## Statistical Analyses

Statistical differences in data among crab mince treatments were evaluated using JMP 7.0.1 (SAS Institute Inc.; Cary, NC) statistical software using one-way analysis of variance (ANOVA) with a significance value of  $p \leq 0.05$ . Differences between means were evaluated using Fisher's least significant difference tests. A multi-way analysis of variance was conducted to evaluate the effects of thermal treatment (boil vs. steam), crab size (carapace width > 55 mm vs. carapace width < 55 mm) and breaker bar setting (0.000 vs. 0.180) on the yield, proximate composition, microbial quality, TVBN content and pH of green crab mince. A correlation analysis was also performed among the dependent variables.

## **Results and Discussion**

### Mechanical Separation

Mechanical separation of green crab was not without difficulties. Very large crabs (carapaces > 65 mm) did not feed into the mechanical separator well. Their large smooth carapaces bounced on the surface of the rotating metal cylinder instead of being pulled into the machine by the cylinder teeth which reduced the feed rate into the hopper. In commercial processing operations, time is a crucial factor and large green crabs may need to be broken into smaller pieces if processed using a Paoli One-Step Deboner Model 22-849.

In addition, when the breaker bar was set to 0.180, as opposed to 0.000, green crabs did not feed well into the feed hopper. The wide open grinding cavity allowed for

a buildup of shell by-product material. When the breaker bar was set to 0.000, there was no room in the grinding cavity for a buildup of crab product. This forced mince through the microslits of the cylinder and ejected shell by-product more efficiently. The buildup seen with the 0.180 breaker bar setting resulted in severe inefficiency. The crab material which was stuck in the grinding cavity was spun around the cylinder and caused overheating of the equipment and product to unacceptable levels. Small smoke trails were noticed coming out of the feed hopper. Shell material which was ejected appeared to have a high concentration of crab mince which indicated that the mince was not being forced through the microslits of the cylinder. Shell discard material was drier and more finely ground when the breaker bar was set at 0.000. Based on these observations, it can be recommended to process smaller crabs at a breaker bar setting of 0.000 using the Paoli One-Step Deboner Model 22-849. Boil versus steam thermal treatments did not have an observable effect on the mechanical processing of the green crab.

### Yield

In food processing operations, determining product yield is a crucial factor in formulating a profitable business plan. Green crab mince yield was determined on a post-cook basis and average yields were 44.7 (BL00), 47.5 (BL18), 51.0 (BS00), 47.3 (SL00), 49.5 (SL18) and 56.1 (SS00) percent (Figure 1). No significant differences ( $p \leq 0.05$ ) in mince yields were noticed between treatments or processing variables based on one-way and multi-way ANOVA. Although not statistically significant, there is an indication that the processing of smaller crabs resulted in higher mince yields.

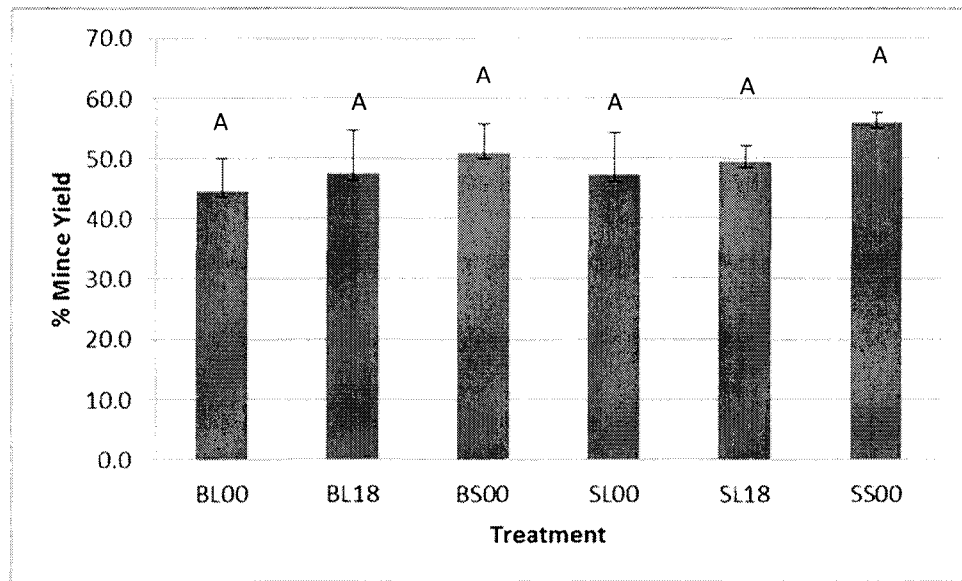


Figure 1 Percent yield on a post-cook basis ( $\pm$  S.D) of green crab mince by treatment. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. BL00 = Boil, Large, 0.000 Breaker Bar; BL18 = Boil, Large, 0.180 Breaker Bar; BS00 = Boil, Small, 0.000 Breaker Bar; SL00 = Steam, Large, 0.000 Breaker Bar; SL18 = Steam, Large, 0.180 Breaker Bar; SS00 = Steam, Small, 0.000 Breaker Bar

On average, hand picking Dungeness and blue crab yields 20-25 % of high quality meat, leaving 75-80 % as by-product. Mechanical processing of crab mince meat from crab shell by-products can recover 15 % meat from discarded by-products while lowering the amount of waste (Gates and Parker, 1992a; Gates and Parker, 1992b; Gates and Parker, 1993). These results would equate to the total extraction of approximately 37 % of useable meat in Dungeness and blue crab processing operations if mechanical separation was used following hand-picking. Average mince meat yield in this study was 49.2 %, supporting the use of whole cooked green crab for mince meat extraction. The shell material by-product was ejected from the mechanical separator

into a finely ground form, which is a prerequisite for the extraction of carotenoid pigments and the production of chitin.

### Moisture Content

In the food industry, determining the total moisture content of foods is fundamental for a variety of processing operations and is one of the most common analytical tests performed. In this study, average moisture contents of mechanically processed green crab mince meat were 82.1 (BL00), 82.2 (BL18), 81.1 (BS00), 80.5 (SL00), 81.1 (SL18) and 81.8 (SS00) percent (Figure 2). No significant differences ( $p \leq 0.05$ ) in moisture content were noticed among treatments or processing variables based on one-way and multi-way ANOVA; however, mince from the boiled large crabs had slightly higher moisture contents.

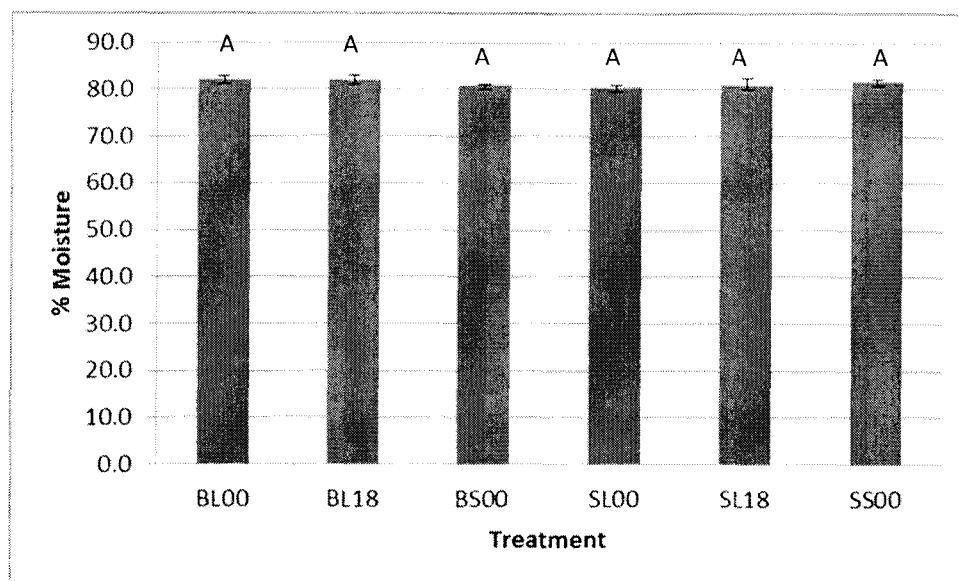


Figure 2 Percent moisture content ( $\pm$  S.D) of green crab mince by treatment. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. BL00 = Boil, Large, 0.000 Breaker Bar; BL18 = Boil, Large, 0.180 Breaker Bar; BS00 = Boil, Small, 0.000 Breaker Bar; SL00 = Steam, Large, 0.000 Breaker Bar; SL18 = Steam, Large, 0.180 Breaker Bar; SS00 = Steam, Small, 0.000 Breaker Bar

The average moisture content of mechanically processed green crab mince meat was 81.5 %. Skonberg and Perkins (2002) studied the proximate composition of green crab leg and claw meat, and reported an average moisture content of 78.7 %. Cherif et al. (2008) also analyzed the moisture content of green crab claw meat and reported results of approximately 79.9-81.2 % total moisture. The results from these studies are similar to this study, although Skonberg and Perkins (2002) and Cherif et al. (2008) analyzed green crab lump meat. Mechanically processed green crab mince has higher ash content. Due to this fact, one would expect a lower moisture content compared to lump meat; however, results from this study show somewhat higher percent moisture in green crab mince. This higher moisture content is likely due to crab viscera contributing

to the total moisture of the mince and the fact that green crabs were cooked. In addition, Baxter (2007) reported an average moisture content of 78.2 % for mechanically processed Jonah crab mince which was comparable to this study.

#### Crude Lipid Content

Understanding the lipid content of green crab mince meat is useful in determining subsequent processing and storage characteristics of value-added products. Average lipid contents were 1.1 (BL00), 1.1 (BL18), 1.8 (BS00), 1.3 (SL00), 1.1 (SL18) and 1.8 (SS00) percent on a wet weight basis (Figure 3). An average lipid content of 1.1 % for large crabs (carapace width >55 mm) and 1.8 % for small crabs (carapace width <55 mm) shows a statistically significant ( $p \leq 0.05$ ) difference between crab sizes (Figure 3). No significant differences in lipid content were noticed between boil and steam thermal treatments or breaker bar setting based on one-way and multi-way ANOVA. The average lipid content among all treatments was 1.4 %.

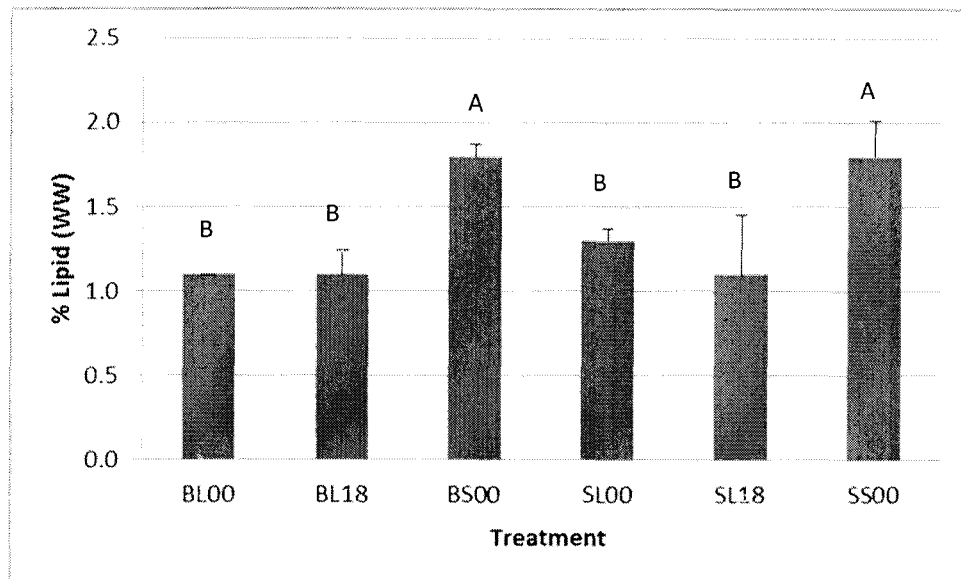


Figure 3 Percent lipid content ( $\pm$  S.D) of green crab mince on a wet weight basis by treatment.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. BL00 = Boil, Large, 0.000 Breaker Bar; BL18 = Boil, Large, 0.180 Breaker Bar; BS00 = Boil, Small, 0.000 Breaker Bar; SL00 = Steam, Large, 0.000 Breaker Bar; SL18 = Steam, Large, 0.180 Breaker Bar; SS00 = Steam, Small, 0.000 Breaker Bar

These results agree with Skonberg and Perkins (2002) who determined the lipid content of green crab leg meat to be 1.2 % and claw meat to be 0.6 %. Naczek et al. (2004) and Cherif et al. (2008) showed average lipid contents of 0.9 % and 0.9 % respectively, which are similar to this study. Cherif et al. (2008) also determined the average lipid content of the hepatopancreas to be 22.3 %. Grinding up the hepatopancreas could be expected to increase the fat content in crab mince compared to crab meat only.

Lipid oxidation is a potential problem in any food containing significant amounts of fat. Although this green crab mince contained an average of only 1.4 % total lipids, oxidative effects may take place during longer periods of frozen storage. In addition,

mechanical separation increases the exposure of the mince to oxygen and metals scraped from the machine which can increase lipid oxidation of omega-3 and other fatty acids.

### Ash Content

The average ash content was 5.1 % for all treatments, with no significant differences ( $p \leq 0.05$ ) among treatments or processing variables based on one-way and multi-way ANOVA; however, the mince from steamed large crabs had slightly higher ash contents than other treatments. Average ash contents were 4.7 (BL00), 5.2 (BL18), 5.0 (BS00), 5.5 (SL00), 5.5 (SL18) and 5.2 (SS00) percent on a wet weight basis (Figure 4). A negative, moderately strong correlation ( $r = -0.77$ ) was revealed between ash and moisture contents ( $p < 0.01$ ). Therefore, when ash content was high, moisture content was low.



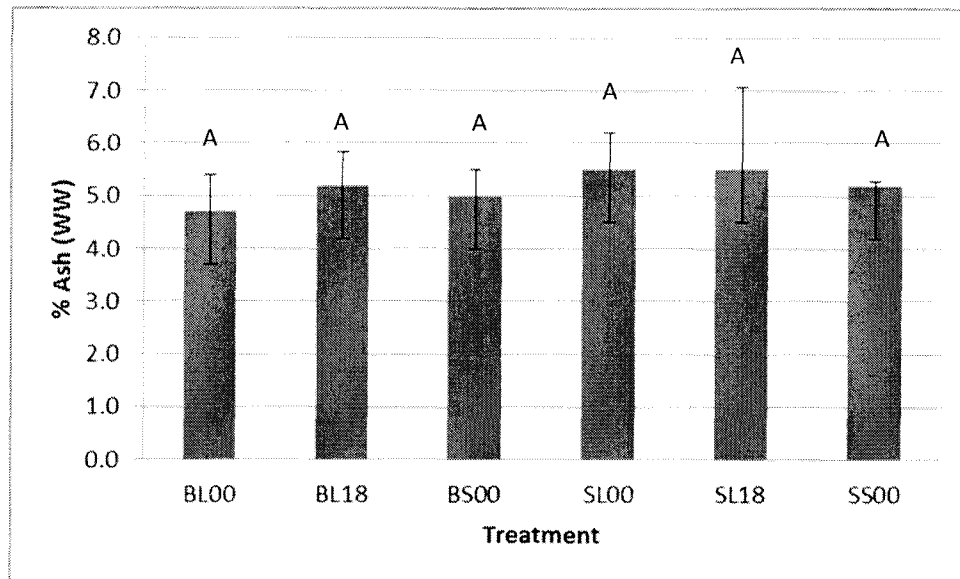


Figure 4 Percent ash content ( $\pm$  S.D) of green crab mince on a wet weight basis by treatment.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. BL00 = Boil, Large, 0.000 Breaker Bar; BL18 = Boil, Large, 0.180 Breaker Bar; BS00 = Boil, Small, 0.000 Breaker Bar; SL00 = Steam, Large, 0.000 Breaker Bar; SL18 = Steam, Large, 0.180 Breaker Bar; SS00 = Steam, Small, 0.000 Breaker Bar

Skonberg and Perkins (2002) evaluated the ash content of green crab meat and concluded an average of 2.2 % on a wet weight basis; however lump meat was used in their study. During the mechanical processing of green crab, some shell particulates transferred through the microslits of the cylinder which resulted in mince with a higher mineral content than that of lump crab meat. Although the processing equipment did an excellent job of removing shell by-product, some shell particulates were transferred into the mince. If green crab meat contains 2.2 % ash (Skonberg and Perkins, 2002) and mechanically processed green crab mince meat contains 5.1 % ash, these results indicate that mechanical processing may double the total mineral content.

Baxter (2007) performed a study on Jonah crab mince gels and found the ash content to be 6.0 % on average; however in Baxter's study, the mince was washed, centrifuged, and water and salts were added to form protein gels, such as seen in the surimi industry. Perhaps the most comparable study was performed by Gillman (2001) who determined the ash content of mechanically separated Jonah crab mince to be 5.8 %. The same Paoli One-Step mechanical separator Model 22-849 (Rockford, IL) was used for this study, with the only differences being the species of crab and the method of introducing the crabs into the feed hopper. Similar ash content was determined between both studies. Total ash content is important in the subsequent value-addition of green crab mince. Too many shell particles can result in a product with a gritty mouthfeel and a decrease in overall consumer acceptability (Gillman, 2001).

#### Crude Protein Content

Results indicate protein contents ranging from 9.7-11.0 %, with an average protein content of 10.4 % for all treatments. Average protein contents were 10.6 (BL00), 9.9 (BL18), 10.7 (BS00), 11.0 (SL00), 10.7 (SL18) and 9.7 (SS00) percent on a wet weight basis (Figure 5). No significant differences in protein content were noticed among treatments or processing variables based on one-way and multi-way ANOVA. A correlation analysis revealed that as % protein decreased, % moisture increased, with a correlation coefficient of -0.62 ( $p < 0.03$ ). No other correlations among variables existed.

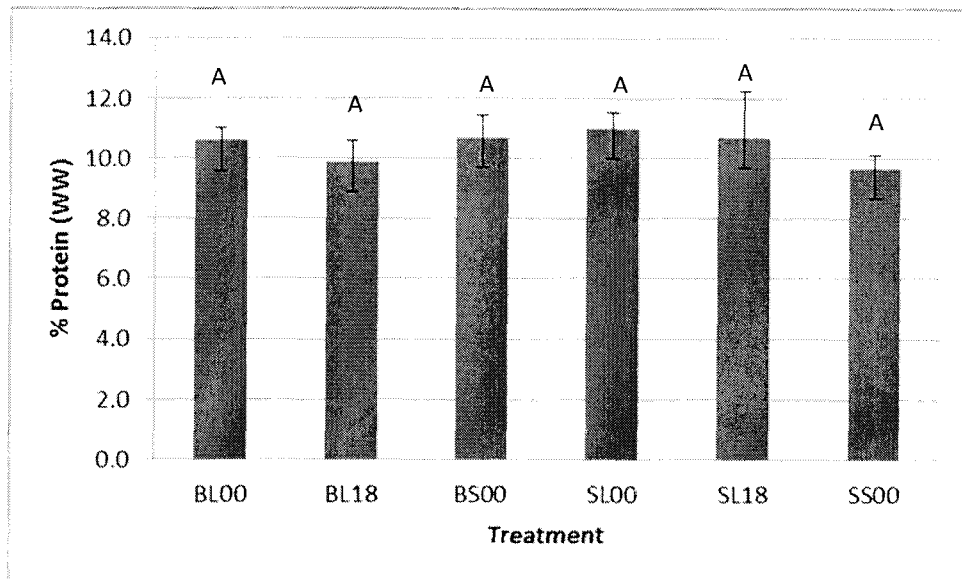


Figure 5 Percent protein content ( $\pm$  S.D) of green crab mince on a wet weight basis by treatment.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. BL00 = Boil, Large, 0.000 Breaker Bar; BL18 = Boil, Large, 0.180 Breaker Bar; BS00 = Boil, Small, 0.000 Breaker Bar; SL00 = Steam, Large, 0.000 Breaker Bar; SL18 = Steam, Large, 0.180 Breaker Bar; SS00 = Steam, Small, 0.000 Breaker Bar

Skonberg and Perkins (2002) studied the nutrient composition of green crab meat and concluded an average of 17.1 % protein on a wet weight basis. Since they evaluated meat instead of processed mince, the results are not directly comparable between studies. It is known that shell material, viscera and other constituents besides the crab muscle do not contribute significantly to overall protein content. It can be assumed if only crab muscle was extracted during mechanical processing, than a strong agreement in protein content would exist between studies. Naczki et al. (2004) also studied the nutrient composition of green crab meat and concluded an average of 82.1 % protein on a dry weight basis. Although protein content on a wet weight basis and

moisture content were not reported, estimations of % protein content on a wet weight basis reveal comparable results among studies.

#### Total Volatile Base Nitrogen (TVBN)

Total volatile base nitrogen (TVBN) level is generally recognized as a freshness indicator and is commonly employed in the seafood industry. In this study, average TVBN values were 16.6 (BL00), 16.9 (BL18), 21.0 (BS00), 18.1 (SL00), 18.2 (SL18) and 17.7 (SS00) milligrams of nitrogen per 100 grams of sample (Figure 6). Treatment BS00 had significantly ( $p \leq 0.05$ ) higher TVBN values than all of the other treatments. No other significant differences in TVBN values among treatments or processing variables were noticed based on one-way and multi-way ANOVA. Average TVBN content was 18.0 mg of N/100 g for all treatments.

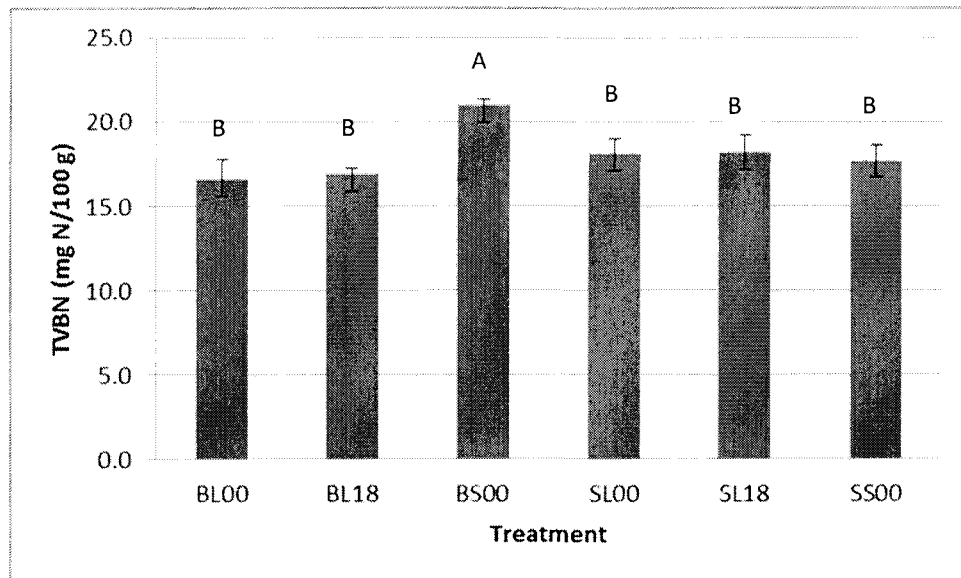


Figure 6 TVBN values ( $\pm$  S.D) expressed as mg N/100 g of green crab mince by treatment. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. BL00 = Boil, Large, 0.000 Breaker Bar; BL18 = Boil, Large, 0.180 Breaker Bar; BS00 = Boil, Small, 0.000 Breaker Bar; SL00 = Steam, Large, 0.000 Breaker Bar; SL18 = Steam, Large, 0.180 Breaker Bar; SS00 = Steam, Small, 0.000 Breaker Bar

Total volatile base nitrogen has long been used as an indicator of the degradation of meat and seafood products. As protein and non-protein nitrogen, trimethylamine oxide, and peptides are broken down because of microbial growth, there is an increase in ammonia and trimethylamines. The European Commission (2005) regards fish products which contain TVBN values exceeding 35 mg N/100 g to be unfit for human consumption. In this study, an average TVBN value of 18.0 mg N/100 g indicates that the fresh, green crab mince was not spoiled by rapid microbiological growth. Gillman (2001) reported the same result of 18 mg N/100 g in mechanically processed Jonah crab mince on day one of an eight day refrigerated storage study.

### Aerobic Plate Counts (APCs)

Average APCs were  $4.3 \times 10^4$  cfu/g for all treatments, and no significant differences in APC values were noticed among treatments or processing variables based on one-way and multi-way ANOVA. Average aerobic plate counts for specific treatments were  $4.5 \times 10^2$  (BL00),  $1 \times 10^3$  (BL18),  $2.5 \times 10^3$  (BS00),  $2.3 \times 10^4$  (SL00),  $2.3 \times 10^5$  (SL18) and  $4.8 \times 10^2$  (SS00) colony forming units (cfu) per gram (Figure 7).

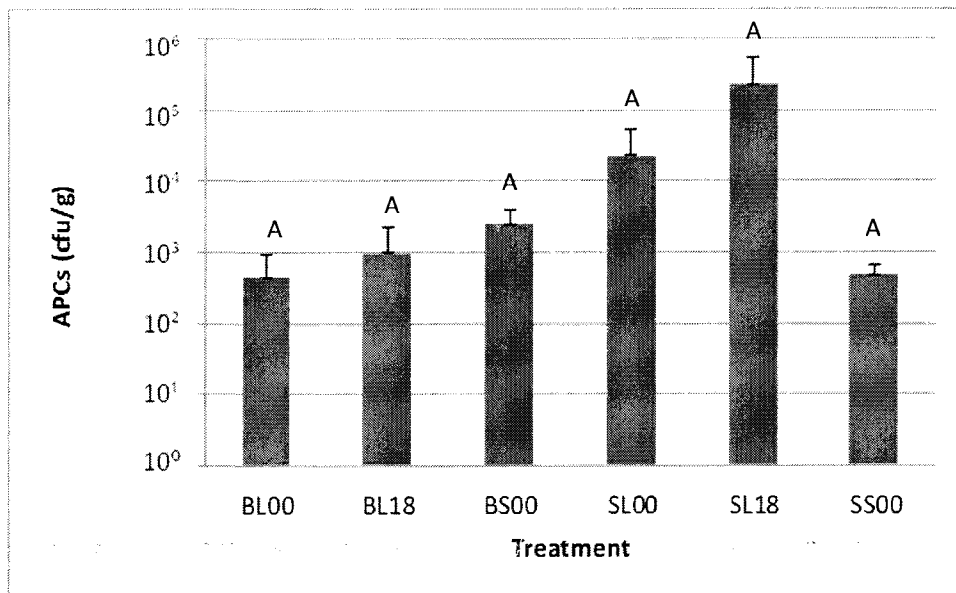


Figure 7 Aerobic plate counts expressed as cfu/g on a logarithmic scale of green crab mince by treatment.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. BL00 = Boil, Large, 0.000 Breaker Bar; BL18 = Boil, Large, 0.180 Breaker Bar; BS00 = Boil, Small, 0.000 Breaker Bar; SL00 = Steam, Large, 0.000 Breaker Bar; SL18 = Steam, Large, 0.180 Breaker Bar; SS00 = Steam, Small, 0.000 Breaker Bar

Variations in APCs occurred among treatments and replications which indicates a breakdown in sanitary and/or analytical procedures. It is most likely that either the

working components of the mechanical separator were not sanitized immediately prior to use, or the collection containers used for collecting the mince were not sanitized properly resulting in the introduction of bacteria during processing and sample collection.

In the seafood industry, raw products with plate counts of  $10^6$ - $10^7$  are generally considered too spoiled for human consumption. Detectable off-odors and flavors are a result of a high spoilage bacterial load which break down proteins into volatile compounds. In this study, although contamination apparently occurred in some batches, APCs were all less than  $2.3 \times 10^5$  cfu/g, with an average of  $4.3 \times 10^4$  cfu/g, indicating that the green crab mince meat was most likely within consumer acceptability tolerances. These values were lower than those obtained by Gillman (2001), who determined aerobic plate counts for mechanically processed Jonah crab mince of  $3.4 \times 10^6$  to  $1.7 \times 10^9$  cfu/g immediately after mechanical processing. A primary reason for the difference among studies was the whole green crabs were processed immediately after cooking, whereas Gillman (2001) separated the crabs into fractions and stored the fractions on ice overnight prior to mechanical separation. In this study, if stricter sanitary controls were followed, estimated APCs of green crab mince would likely have ranged from  $10^3$ - $10^4$  cfu/g which indicates green crab mince to be of good microbiological quality. Low APCs are further substantiated by low TVBN values and non-detectable levels of yeasts and molds during preliminary analyses.

### Color (L\*a\*b\*)

In the food industry, color is one of the most important quality factors in determining consumer acceptability. Average L\*a\*b\* values were 52.2, 5.6, 14.7 (BL00); 52.3, 6.2, 14.6 (BL18); 49.0, 7.5, 13.9 (BS00); 52.1, 6.2, 15.2 (SL00); 51.4, 6.6, 15.1 (SL18); and 49.8, 6.6, 13.9 (SS00) (Figure 8). Average L\*a\*b\* values for all treatments were 51.1, 6.5 and 14.6. Average L\* values of large crabs (52.0) and small crabs (49.4) indicated a significant difference between crab sizes (Figure 8). Average a\* values were 6.2 (large crabs) and 7.1 (small crabs), while average b\* values were 14.9 (large crabs) and 13.9 (small crabs).

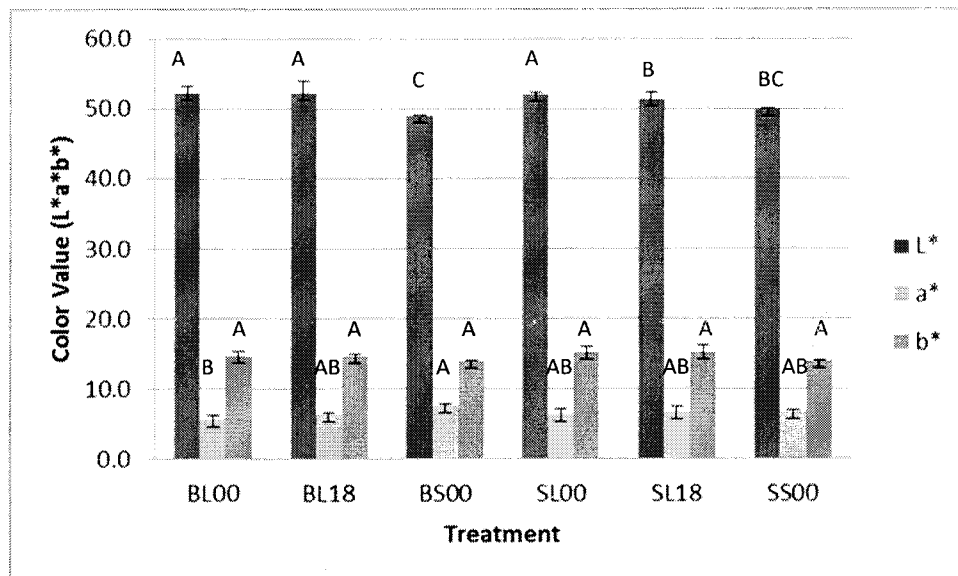


Figure 8 L\*a\*b\* values ( $\pm$  S.D) for color of green crab mince by treatment. L\*a\*b\* values not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. BL00 = Boil, Large, 0.000 Breaker Bar; BL18 = Boil, Large, 0.180 Breaker Bar; BS00 = Boil, Small, 0.000 Breaker Bar; SL00 = Steam, Large, 0.000 Breaker Bar; SL18 = Steam, Large, 0.180 Breaker Bar; SS00 = Steam, Small, 0.000 Breaker Bar



L\* values indicate a range of black (0) to white (100). The a\* values indicate a range of negative numbers, which represent green colors, to positive numbers, which represent red colors (Nielson, 2010). The b\* values indicate a range of negative numbers, which represent blue colors, to positive numbers, which represent yellow colors. These values are used to approximate human vision. In the restructured seafood and surimi industries, whiter color of fish mince equates to higher quality and subsequently more profit from sales. Using the L\*a\*b\* scale for colorimetric analysis is a quick method for gaining quantitative insight on the color of a product.

Visually, green crab mince meat from this study can be described as having a light 'carroty-brown' color. Tiny white specks were seen in the mince due to the incorporation of shell particles during mechanical processing. Baxter (2007) performed colorimetric analysis on Jonah crab mince gels and determined L\*a\*b\* values of approximately 61.2, 13.9 and 21.5 in unwashed control treatments. A comparison between studies shows that Jonah crab mince gels were whiter and contained more reddish-yellowish hues than green crab mince meat. Baxter (2007) washed the crab mince several times which was likely a factor in increasing the whiteness in Jonah crab mince gels. Interestingly, the green crab results show that smaller green crabs have significantly higher lipid contents and significantly lower L\* values than their larger counterparts. The correlation coefficient between lipid content and L\* color value was -0.79 ( $p < 0.01$ ) and the correlation coefficient between lipid content and b\* color value was -0.77 ( $p < 0.01$ ) which represent strong correlations between values. The color of

extracted lipids from green crab was a dark orange color possibly due to the content of fat soluble carotenoid pigments.

## pH

pH is a measure of the acidity or alkalinity of a solution or food, and is a factor in the control of spoilage and pathogenic microorganisms. Average pH was 8.1 (BL00), 8.1 (BL18), 8.0 (BS00), 8.1 (SL00), 8.1 (SL18) and 8.1 (SS00), with an average of 8.1 for all treatments. A pH below 4.6 generally provides unfavorable conditions for microbial growth. A pH of 8.1 generally supports the growth of all microorganisms of spoilage and pathogenic concern in food. Hurdle techniques used to reduce or eliminate the prevalence of bacteria should be incorporated in the mechanical processing of green crab. Green crab mince should be frozen or refrigerated, and incorporated quickly into value-added food products.

## **Conclusions**

This study focused on the mechanical separation of green crab and its effects on crab mince meat yield and quality. A high mince meat yield indicates that the mechanical extraction of green crab mince meat has the potential to be economically viable in commercial processing operations. Although not statistically significant, small crabs (<55 mm carapace width) had a slightly higher yield than large crabs (>55 mm carapace width). In addition, small crabs had a significantly ( $p \leq 0.05$ ) higher lipid content than large crabs; however, both treatments contained low amounts of total

lipids which indicate little concern for oxidative effects during long-term frozen storage. L\* color value of mince meat was also significantly ( $p \leq 0.05$ ) affected by crab size, and negative correlations with lipid content indicated that fat soluble carotenoid pigments may have influenced the color of green crab mince meat. Although mechanical processing was effective in removing shell by-product, some shell material was unavoidable in the mince which resulted in an increased mineral content compared to whole meat, which may negatively impact the consumer acceptability of value-added crab mince products. Low TVBN and APC values indicate that green crab mince meat is of good microbial quality and acceptable for human consumption. Proximate analyses show green crab mince meat to be a good source of protein which is a factor in the marketability and subsequent processing of the mince, and an essential nutrient in the human diet. Mechanical processing difficulties at the 0.180 breaker bar setting indicate that a tighter grinding cavity is preferable. Overall results indicate that small green crabs which were mechanically processed at a 0.000 breaker bar setting, regardless of thermal treatment, resulted in the highest mince yield and were easiest to process.

### CHAPTER 3. DEVELOPMENT AND CONSUMER ACCEPTABILITY OF EMPANADAS CONTAINING GREEN CRAB (CARCINUS MAENAS) MINCE MEAT

#### Objectives

The overall objective of this study was to develop a consumer acceptable empanada containing green crab mince meat, and the specific objective was to determine consumer acceptability of empanadas containing additions of 30, 50 and 70 % green crab mince by filling weight.

#### Materials and Methods

##### Experimental Design

In this study, three different empanadas were developed and evaluated by consumers. Each empanada contained different quantities of green crab mince meat; specifically 30, 50 or 70 % of filling weight (Table 2), which was the only variable among treatments. The three empanada formulations were evaluated for acceptability using a consumer sensory panel.

Table 2 Empanada Treatments

Treatment	% Crab Mince Meat in Filling
1	30
2	50
3	70

### Product Concept

Traditionally, new products are developed based on consumer trend analysis. Most consumer demand is for convenient, flavorful, healthy, safe and cost-efficient products which may or may not include special dietary considerations. Since the overall objective of this specific study was to develop a new food product containing a significant amount of green crab mince, preliminary consumer trend analyses were not conducted.

Empanadas were chosen for product development because they can contain large amounts of green crab mince meat. In addition, they are inexpensive to produce and may be accepted by the growing Latino-American population within the United States. Other products considered for this study were; crab cakes, tortillas, quiches, tamales, crab dogs and fondues.

An empanada is a South American stuffed and fried pastry which is the national dish of Argentina. It involves wrapping small circular cuts of dough around fillings which traditionally contain chicken, beef or pork along with a variety of fillers such as beans, rice, potatoes, onions or eggs. Empanadas are also known as pastelitos, which directly translates from Spanish to 'little cakes' or 'little pies'.

### Empanada Processing

To prepare the green crab mince meat, mixed sized green crabs were boiled to an internal temperature of at least 74° C, cooled in an ice bath and processed using a Paoli One-Step mechanical separator Model 22-849 (Rockford, IL) with a breaker bar

setting of 0.000. Green crab mince was immediately transferred into large 3 mil Ultravac (Ultravac Solutions LLC.; Kansas City, MO) polyethylene bags and vacuum sealed using a Koch Ultravac Model UV 550 (Koch Equipment LLC.; Kansas City, MO) which removed 80 % of the air inside the bag. Bags were then transferred into a blast freezer for at least 30 minutes where the ambient temperature was approximately -34° C. Once the mince was frozen solid, it was held in frozen storage (<0° C) for approximately two weeks.

Green crab mince was thawed at refrigeration temperatures (~4° C) for 48 hours before use. Excess water was then removed from the crab mince by hand pressing in a colander. The three empanada treatments (Table 2) were prepared in the Department of Food Science and Human Nutrition's Commercial Kitchen according to the following protocol. A large sauté pan was heated on medium heat with a small amount of vegetable oil. Spanish onion and red pepper were diced to ⅛ of an inch (i.e. brunoise) and added to the sauté pan along with fresh corn kernels. The vegetables were cooked for approximately five minutes to allow for a slight caramelization reaction to occur in the vegetables. Dewatered crab mince, thyme and cayenne pepper were then added in the sauté pan, the heat was turned to low and the mixture was allowed to cook to a temperature of 74° C.

The dough for all three treatments was prepared as follows. In a KitchenAid bowl mixer Model KM25G0XWH (Dayton, OH); bleached flour (Gold Medal Products Co.; Cincinnati, OH), double acting baking powder (Rumford Chemical Works; Terre Haute, IN) and salt were mixed together. A small amount of vegetable shortening (J.M. Smucker Co.; Orrville, OH) was then added to the dry ingredients until the mixture had a

sandy resemblance. Warm tap water was added to an electric bowl mixer with a paddle attachment and the dry ingredients were slowly added to form the dough. Once the dough was formed, care was taken as not to overwork it. It was formed into a ball by hand and allowed to stand for 10 minutes. After 10 minutes, the dough was rolled out until almost translucent (~½ mm thickness) and cut using a 3 inch diameter circular cookie cutter. The dough circles were verified to weigh between 10-12 grams each.

To produce the empanadas, approximately 7.4 grams of cooled filling was placed into the center of each dough circle. The edges of the dough circles were wetted; one end of the dough was pulled over the filling, and the empanadas were sealed. Each uncooked empanada weighed approximately 18 grams. Empanadas were arranged on half-sheet pans layered with parchment paper (Reynolds®; Richmond, VA) and placed under frozen temperatures (<0° C) for approximately 8 hours. One-hundred empanadas from each of the three treatments were produced.

All of the ingredients (Table 3) were purchased at Hannaford Bros. Co. in Old Town, ME with the exception of the crab mince. The list of ingredients and exact method of preparation can be seen in the recipe (Appendix A). Preliminary value-added product development was performed using Jonah crab mince from Portland Shellfish Co. (Portland, ME).

Table 3 Empanada Formulations

	<i>Ingredients</i>	<i>Treatment 1</i> 30 %	<i>Treatment 2</i> 50 %	<i>Treatment 3</i> 70 %
	Vegetable Oil (For Frying)	As needed	As needed	As needed
<i>For Dough:</i>	AP Flour	156 g	156 g	156 g
	Salt	4 g	4 g	4 g
	Double Acting Baking Powder	2 g	2 g	2 g
	Vegetable Shortening	40 g	40 g	40 g
	Water (Warm)	65 g	65 g	65 g
<i>For Filling:</i>	Crab Mince	54 g	126.4 g	295 g
	Spanish Onion (Diced)	38 g	38 g	38 g
	Corn Kernels (Fresh)	50 g	50 g	50 g
	Red Pepper (Diced)	38 g	38 g	38 g
	Thyme (Dried)	0.2 g	0.2 g	0.2 g
	Cayenne Pepper (Dried)	0.2 g	0.2 g	0.2 g
	Water	As needed	As needed	As needed



Figure 9 Green crab and vegetable empanada.



## Sensory Analysis

Sensory analysis of green crab empanadas was conducted at the Department of Food Science and Human Nutrition's Consumer Testing Center and consisted of eighty-seven panelists who tasted three empanada samples each. An Institutional Review Board (IRB) application and supporting documents were submitted to the Human Subjects Protection Committee of the University of Maine, and this study was approved for gathering data from human panelists. All panelists were recruited by flyer (Appendix B) or e-mail postings and were between the ages of 18 and 65. They were presumed to enjoy crab products and were asked not to participate if they had allergies to seafood, soy and/or wheat. SIMS 2000 software version 6 (Sensory Computer Systems; Morristown, NJ) for Windows was used to generate the questionnaire as well as collect data. After reading an informed consent form (Appendix C) and signing in, panelists were seated in booths with partitions in a room with positive-pressure air flow, as to prevent bias judgments from odors in the preparation area. Both fluorescent and incandescent lighting were used. Empanadas, which had been partially defrosted under refrigeration temperatures ( $\sim 4^{\circ}\text{C}$ ) for approximately 2 hours, were shallow fried in vegetable oil for 3 minutes on each side, cut in half and held on a Duke® Model ACTW-I M (St. Louis, MO) steam table for no longer than 10 minutes. Half-empanada samples were served warm ( $\sim 35^{\circ}\text{C}$ ) on pre-coded paper plates and trays, to panelists in a random order using randomly generated sample codes for identification. Samples were accompanied by napkin and a cup of water to rinse residual flavors away between samples. Instructions were given to the panelists by a computerized ballot to guide

them through the sensory test. Although panelists were asked to sign in, the collection of data was performed anonymously.

Panelists were first asked demographic questions including: what is your gender, what is your age, how often do you consume crab or products containing crab, what form do you most often consume crab, in what setting do you most often consume crab products, and what is your most preferred type of food? One half of the three different green crab and vegetable empanadas were then served to each panelist for evaluation. Panelists were asked to evaluate the appearance, filling appearance, texture, flavor and overall acceptability of each empanada based on the 9-point hedonic scale developed by Peryam and Pilgrim (1957) (Table 4). Panelists were then asked: what improvements would you make to the empanadas you just tasted, and if a product similar to the empanadas you just tasted was available to you locally, how likely would you be to purchase it? The former question was open-ended while the latter was based on a 4-point scale (i.e. definitely would not buy; probably would not buy; probably would buy; definitely would buy). An open-ended section for additional comments marked the conclusion of the questionnaire (Appendix D).

Table 4 9-point Hedonic Scale Used to Evaluate Empanada Attributes

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

## Statistical Analyses

Data was collected using SIMS 2000 version 6 software (Sensory Computer Systems; Morristown, NJ) for Windows®. Data was analyzed for differences in means using SAS version 9.2 (SAS Institute Inc.; Cary, NC). Raw data was transcribed into JMP 7.0.1 (SAS Institute Inc.; Cary, NC) statistical software and verified for accuracy against the SIMS 2000 data. A multi-way analysis of variance (ANOVA) was conducted to evaluate significance among empanada attributes and demographics. A correlation analysis was performed on the empanada attributes using JMP 7.0.1 (SAS Institute Inc.; Cary, NC).

## **Results and Discussion**

### Sensory Analysis

Determining the consumer acceptability of food products using a sensory panel is an essential component of the product development process. Of the total 87 panelists, 45 were female, 32 were male and 10 provided no answer. Approximately half of the respondents were between the ages of 18-30 while the other half were between the ages of 31-65. Sixty percent of the panelists eat crab, or products which contain crab, 'every few months', with only 21 % reported consuming crab at least once per month (Figure 10). One-third of the panelists reported that crab cakes were the major form of crab intake in their diet. Chowders/soups, whole crabs and fried appetizers were also major sources of crab intake in the panelists' diets (Figure 11). Over 60 % of panelists eat crab products in restaurant settings the majority of the time (Figure 12).

Interestingly, no one selected 'home' as the setting most often used for the consumption of crab products. Favorite food ethnicities included American, followed by Italian, Mexican, Chinese and Indian.

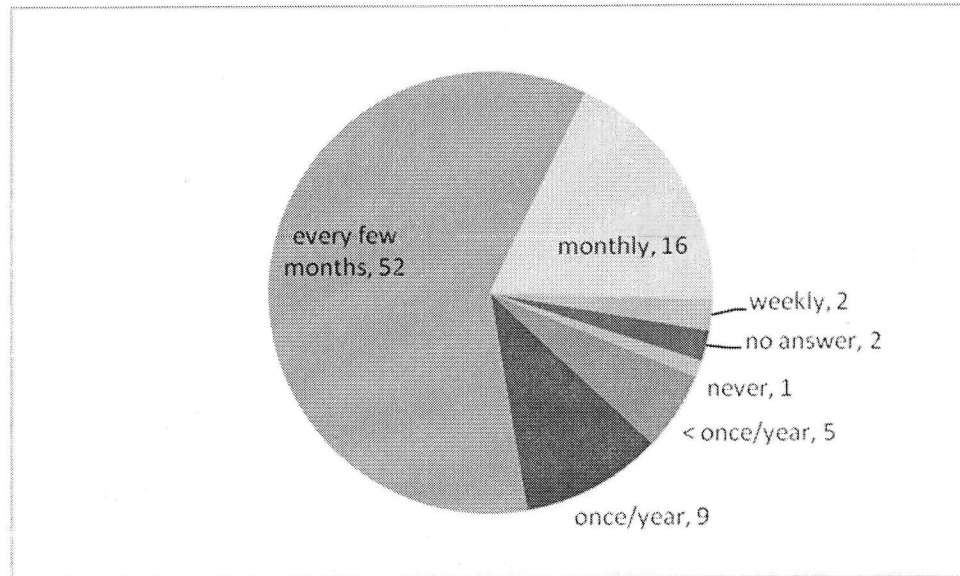


Figure 10 Consumer response to the question; 'How often do you consume crab or products containing crab?' Numbers represent number of panelists. n = 87

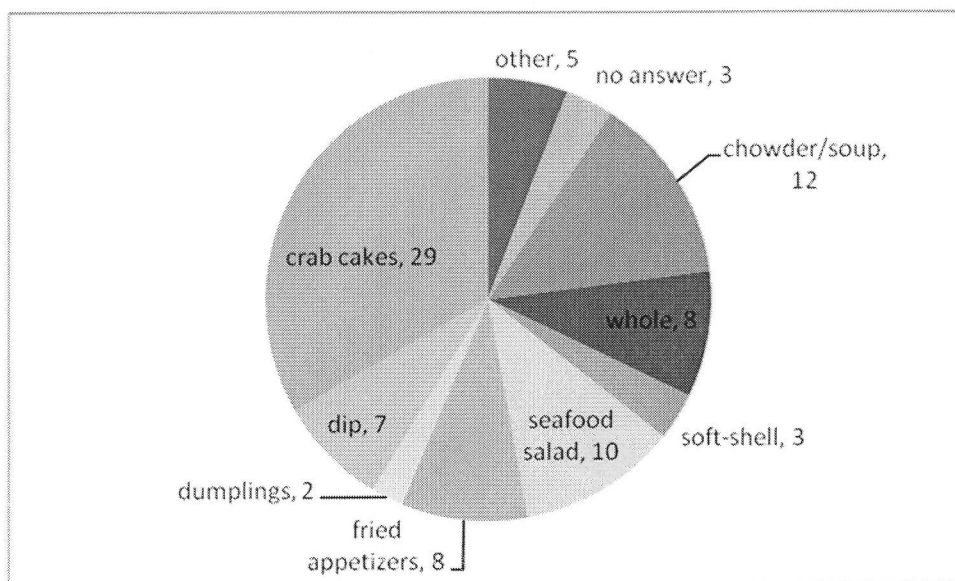


Figure 11 Consumer response to the question; 'In what form do you most often consume crab?' Numbers represent number of panelists. n = 87

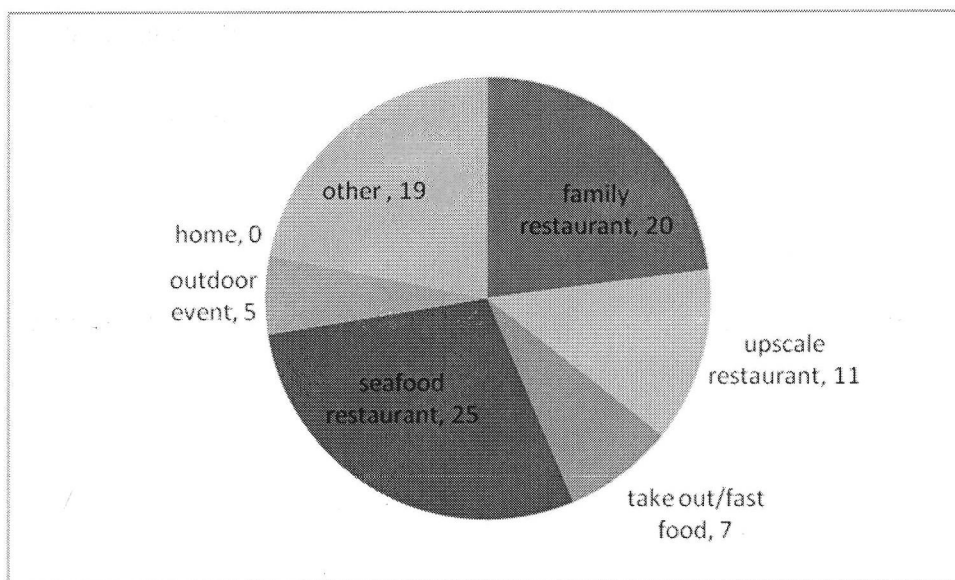


Figure 12 Consumer response to the question; 'In what setting do you most often consume crab products?' Numbers represent number of panelists. n = 87

Empanada attributes of appearance and filling appearance received mean panelist scores of 6.6, 6.1 (30 %), 6.6, 6.2 (50 %), 6.4 and 5.6 (70 %) (Figure 13). Filling appearance was significantly ( $p \leq 0.05$ ) less liked in the 70 % samples compared to the empanadas containing 30 and 50 % mince meat based on one-way ANOVA. Although not statistically significant, there is a noticeable decrease in hedonic scores for appearance in the 70 % samples compared to the empanadas containing 30 and 50 % mince meat. Panelist comments indicated that the empanadas were under-filled which may have reduced the hedonic scores for the attributes of appearance and filling appearance. The under-filled appearance was most likely due to the baking powder which acted during shallow frying and released carbon dioxide into the empanadas. This release of carbon dioxide inflated the empanadas and gave the appearance that they were under-filled. Reformulation may consist of reducing, or omitting entirely, baking powder in the recipe. Other comments mentioned that the empanadas were too dark, indicating that they were cooked for too long; however, the reason for significant differences in appearance scores among treatments is unclear.

The empanada attribute of texture received mean panelist scores of 6.7 (30 %), 6.8 (50 %) and 6.4 (70 %) (Figure 13). Although not statistically significant, there is a noticeable decrease in hedonic scores for texture in the 70 % samples compared to the empanadas containing 30 and 50 % mince meat. Panelist comments indicate variability in how well they liked the texture of the empanadas. Although some panelists commented that the texture was to their liking, many mentioned that the crust was too flaky and not crispy enough. Reformulation may consist of using flour which contains

higher gluten content and/or kneading the dough for a longer period of time. These techniques would form dough with tighter gluten bonds and subsequently an empanada crust which is less flaky and chewier.

Mean empanada flavor scores were 6.8 (30 %), 6.7 (50 %) and 6.2 (70 %) (Figure 13). Flavor was significantly ( $p \leq 0.05$ ) less liked in the 70 % samples compared to the empanadas containing 30 and 50 % mince meat. Flavor is often the most powerful sensory attribute which contributes to the overall acceptability of a food product. In this study, a strong correlation existed between flavor and overall acceptability with a correlation coefficient of 0.91 ( $p < 0.01$ ). Panelist comments indicated that the empanadas lacked crab flavor. Green crab mince used in the empanada formulations was pre-cooked, and frozen and thawed twice, which resulted in a decrease in final crab flavor. In addition, the green crab visceral components, which were a part of the mince, may have contributed to off-flavors in the empanadas. Off-flavors could be masked with the additions of a sauce and/or crab flavorings. Comments further indicated that empanada samples which contained 50 % crab mince by filling weight had the best balance of flavor among the mince, vegetables and seasonings.

Overall acceptability received mean panelist scores of 6.8 (30 %), 6.8 (50 %) and 6.1 (70 %) (Figure 13). The overall acceptability was significantly ( $p \leq 0.05$ ) lower in the 70 % samples compared to empanadas containing 30 and 50 % mince meat based on one-way ANOVA. Panelist comments indicated that they wanted a green crab appetizer which wasn't so greasy and unhealthful. Other panelists commented that the

empanadas were too dry which could be resolved with the addition of a small amount of sauce.

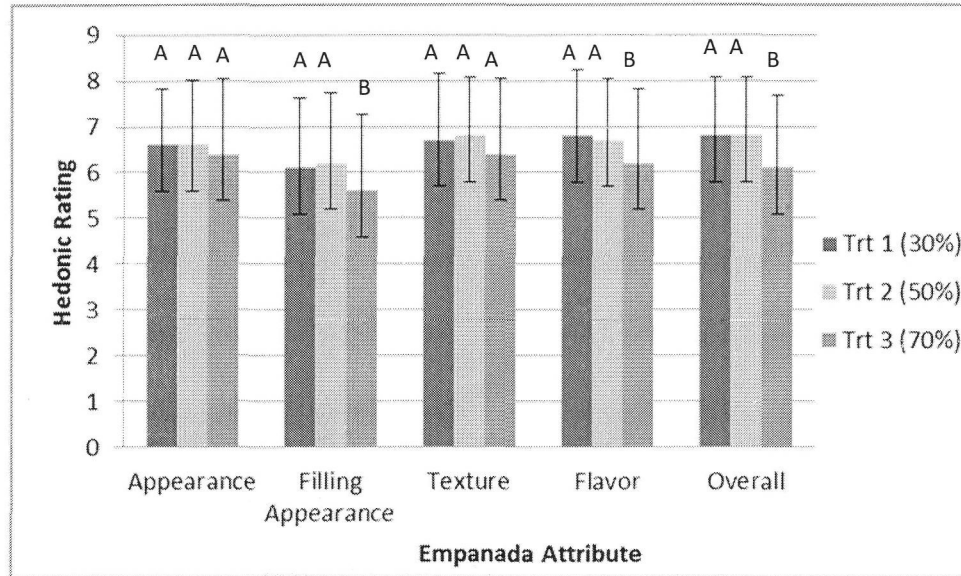


Figure 13 Hedonic scores of green crab and vegetable empanada attributes ( $\pm$  S.D) by treatment.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) within each attribute based on one-way ANOVA.  $n = 87$

These sensory results equate to overall consumer acceptability of 'like slightly' (i.e. hedonic score of 6) to 'like moderately' (i.e. hedonic score of 7) with the exception of filling appearance in the 70 % samples which was between 'neither like nor dislike' (i.e. hedonic score of 5) and 'like slightly'. Hedonic ratings above 7 are generally considered promising for commercial production. The results from this study are somewhat comparable to Gillman (2001) who performed sensory analysis on an extruded Jonah crab mince pasta product. Gillman (2001) reported that pasta containing 10-20 % crab mince received consumer acceptability scores of 'like slightly' to 'like



moderately'; however, the studies were researching two different value-added products.

Near the conclusion of the consumer questionnaire, participants were asked if they would purchase the empanadas if available to them locally (Figure 14). Results showed that 11.5 % of participants would 'definitely buy', 51.7 % would 'probably buy', 32.2 % would 'probably not buy' and 4.6 % would 'definitely not buy' the empanadas, which indicates promise for future value-added products containing green crab mince.

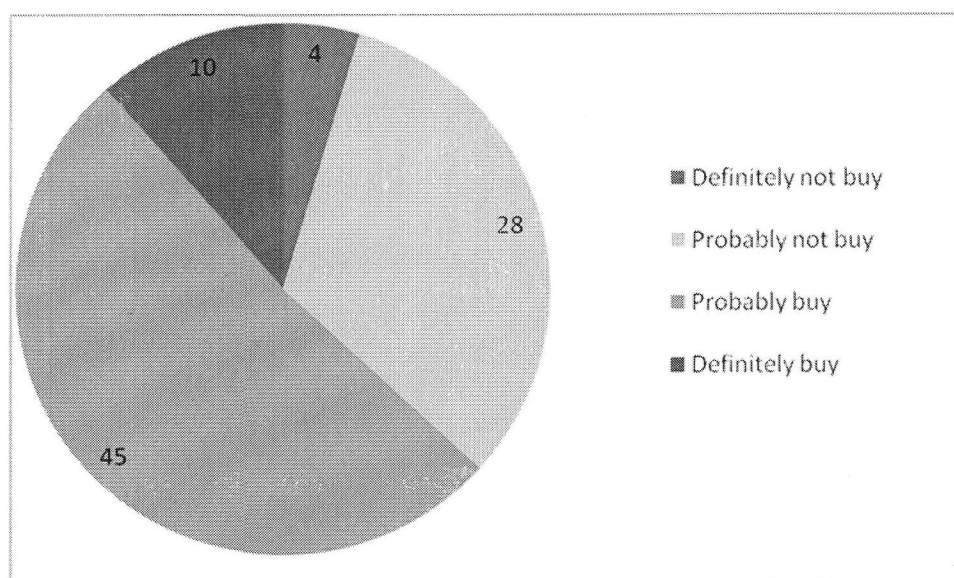


Figure 14 Consumer response to the question; 'If a product similar to the empanadas you just tasted was available to you locally, how likely would you be to purchase it?'  
Numbers represent number of panelists. n = 87

A multi-way ANOVA revealed no significant differences ( $p \leq 0.05$ ) in empanada attribute scores based on gender, age, rate and type of crab consumption or favorite food ethnicity. Results indicate that as the percentage of green crab mince increased in the empanada formulation from 50 to 70 %, overall consumer acceptability decreased.

Attribute scores above 'like slightly' and 63.2 % of panelists who indicated that they 'probably or definitely would buy' indicate promise for future green crab mince meat product development.

When developing new food products, large scale processors typically perform more than one sensory test before product launch. Sensory tests allow producers to understand what consumers prefer, as opposed to 'pushing' a product into the market. Overall, this study has provided a solid platform for green crab mince meat product development. Hedonic scores showed green crab empanadas to be somewhat liked, however there is still room for improvement. Reformulation and further sensory analysis would be the next logical steps in the development of a green crab mince meat product.

## **Conclusions**

In this study, panelists were recruited to determine the consumer acceptability of green crab and vegetable empanadas containing different levels of crab mince meat, specifically 30, 50 and 70 % crab mince by total filling weight. Statistical analysis indicated that empanadas containing 70 % mince meat were significantly ( $p \leq 0.05$ ) less liked in the attributes of filling appearance, flavor and overall acceptability compared to the 30 and 50 % empanadas. Consumer acceptability scores for all attributes were between 'like slightly' and 'like moderately' with the exception of filling appearance in the 70 % samples which was rated between 'neither like nor dislike' and 'like slightly'. Over sixty-three percent of panelists indicated that they 'probably or definitely would

buy' green crab and vegetable empanadas if they were available locally. Comments indicated that panelists would have preferred a more healthful, fuller, moister, more flavorful empanada with a less flaky crust. Future development of green crab mince empanadas should explore the addition of a sauce and/or flavoring agent. Overall attribute scores approaching 'like moderately' (i.e. hedonic score of 7) for the 30 and 50 % empanadas, and strong willingness to purchase results indicate promise for green crab mince empanadas, however further product development is necessary to improve consumer acceptability of this novel product.

## CHAPTER 4. EFFECTS OF TRANSGLUTAMINASE, ISOLATED SOY PROTEIN AND DRIED EGG WHITE ON TEXTURE, COLOR, COOK YIELD, WATER-HOLDING CAPACITY, AND PROXIMATE COMPOSITION OF GREEN CRAB (*CARCINUS MAENAS*) MINCE PATTIES

### Objectives

This study was performed in two experiments. The objective of the first experiment was to determine how additions of 1, 2 and 4 % transglutaminase affected the textural profile, color ( $L^*a^*b^*$ ), cook yield, water-holding capacity and proximate composition of fully cooked green crab (*Carcinus maenas*) mince meat patties. The objective of the second experiment was to determine how additions of 5 % isolated soy protein, 5 % dried egg white and their combinations with 2 % transglutaminase affected the textural profile, color ( $L^*a^*b^*$ ), cook yield, water-holding capacity and proximates of fully cooked green crab (*Carcinus maenas*) mince patties.

### Materials and Methods

#### Preliminary Patty Development

The first step in the development of green crab mince patties containing restructuring additives was to determine the experimental levels of transglutaminase, isolated soy protein and dried egg white to add to the green crab mince meat. Due to the fact that green crab mince is a previously cooked product, which contains denatured proteins, it was decided to increase the levels of restructuring additives to compensate for lower functionality compared to raw meat systems. In addition, based on supplier

recommendations, pure transglutaminase was not recommended for this application. Instead, a transglutaminase preparation called Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) was used. It contains sodium chloride, gelatin, trisodium phosphate, maltodextrin, safflower oil and 0.65 % pure transglutaminase on a per weight basis.

Two separate preliminary tests were performed. The first test was designed to determine the effectiveness of different levels of Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation at improving the textural characteristics of green crab mince patties (Figure 15). It included 0.5, 1, 2, 4 and 8 % transglutaminase added to green crab mince meat. The second test was designed to determine the effectiveness of different levels of Ardex® F Dispersible isolated soy protein (ADM Specialty Food Ingredients Division; Decatur, IL) and Deb El™ Just Whites (Deb-El Foods Corp.; Elizabeth, NJ) dried egg white, specifically 5 and 10 % additions each (Figure 16). The results from these cook tests were interpreted qualitatively and used to determine the optimum levels of restructuring additives to be used in subsequent experimentation. It was determined that the first experiment would test the effectiveness of 1, 2 and 4 % transglutaminase additions, and the second experiment would test the effectiveness of 5 % isolated soy protein, 5 % dried egg white and their combinations with 2 % transglutaminase on green crab mince meat patties.

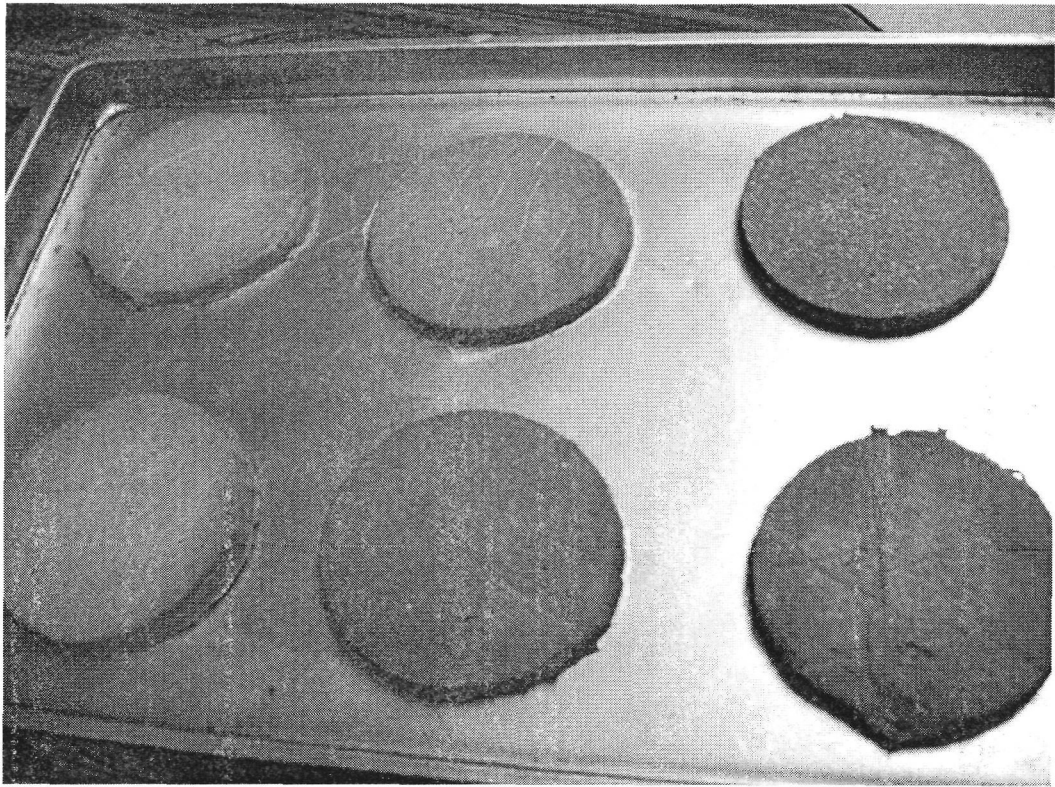


Figure 15 Fully cooked green crab patties containing different levels of transglutaminase.  
(top row from left – 0.5, 2, 8 %; bottom row from left – 0, 1, 4 %)

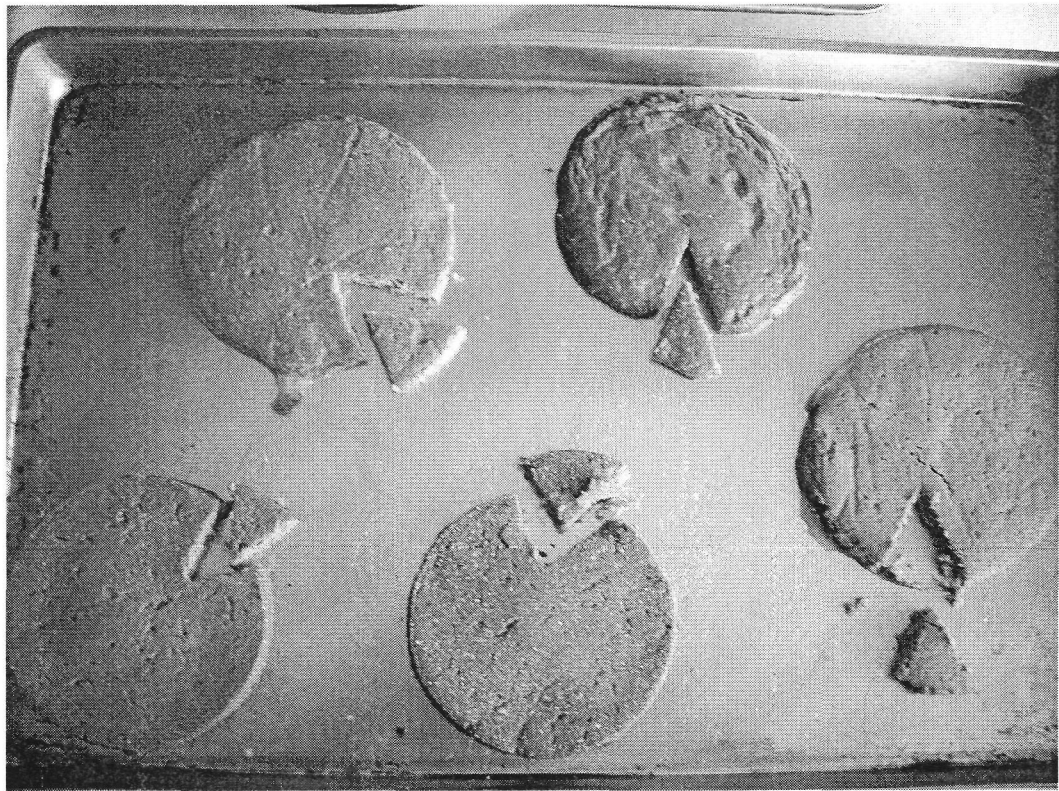


Figure 16 Fully cooked green crab patties containing different levels of isolated soy protein and dried egg white.

(clockwise from top left – 5 % egg, 10 % egg, no additives, 10 % isolated soy protein, 5 % isolated soy protein)

#### Mince Meat Preparation

Mixed sized green crabs were boiled to an internal temperature of at least 74° C, cooled in an ice bath and processed using a Paoli One-Step mechanical separator Model 22-849 (Rockford, IL) with a breaker bar setting of 0.000. Green crab mince was immediately transferred into a large polyethylene bag (Ultravac Solutions LLC.; Kansas City, MO) and vacuum sealed using a Koch Ultravac Model UV 550 (Koch Equipment LLC.; Kansas City, MO) removing 80 % of the air inside the bag. The bag was then transferred into a Bally PowerBlast® blast freezer (BMIL International Inc.; Morehead

City, NC) for at least 30 minutes where the ambient temperature was approximately - 34° C and subsequently held under frozen temperatures (~-17° C) until further use.

Before the frozen green crab mince was used for patty formation, it was necessary to remove excess water. The crab mince was defrosted in a True® refrigerator (True Manufacturing Co.; St. Louis, MO) ( $5^{\circ}\text{C} \pm 1^{\circ}$ ) and the mince was pressed by hand against a fine mesh screen. This dropped the moisture content of the green crab mince from ~81 % to ~75 %. The green crab mince was then mixed well and distributed into six polyethylene bags which corresponded to each batch within each experiment. The green crab mince meat was then re-frozen using the blast freezer and held under frozen temperatures until further use.

#### Experiment 1

The first of the two experiments in this study was designed to determine how additions of 1, 2 and 4 % Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation additions affected the textural profile, color ( $L^*a^*b^*$ ), cook yield, water-holding capacity and proximate composition of patties made from fully cooked green crab mince. The treatment codes and patty formulations for the first experiment can be seen in Table 5. Each treatment was prepared in triplicate batches and each batch yielded two 100.0 gram patties for a total of six patties per treatment.



Table 5 Treatment Codes and Formulations Used in the Experimental Design for the First Experiment of this Study

Treatment	Code	Green Crab Mince (g)	Salt (g)	Activa® GS Transglutaminase Preparation (g)
1	C	222.75	2.25	0.00
2	T1	220.50	2.25	2.25
3	T2	218.25	2.25	4.50
4	T4	213.75	2.25	9.00

C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

## Experiment 2

Experiment two was designed to determine how additions of 5 % isolated soy protein, 5 % dried egg white and their combinations with 2 % Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation affected the textural profile, color ( $L^*a^*b^*$ ), cook yield, water-holding capacity and proximate composition of fully cooked green crab mince meat patties. The treatment codes and patty formulations for the second experiment can be seen in Table 6. As with the first experiment, each treatment was prepared in triplicate batches and each batch yielded two 100.0 gram patties for a total of six patties per treatment.

Table 6 Treatment Codes and Formulations Used in the Experimental Design for the Second Experiment of this Study

Treatment	Code	Green Crab Mince (g)	Salt (g)	Activa® GS Transglutaminase Preparation (g)	Isolated Soy Protein (g)	Dried Egg White (g)
1	C	222.75	2.25	0.00	0.00	0.00
2	I	211.50	2.25	0.00	11.25	0.00
3	E	211.50	2.25	0.00	0.00	11.25
4	TI	207.00	2.25	4.50	11.25	0.00
5	TE	207.00	2.25	4.50	0.00	11.25

C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 %

Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

#### Patty Preparation

To begin the formulations, the previously pressed and frozen green crab mince was defrosted under refrigeration temperatures ( $5^{\circ}\text{C} \pm 1^{\circ}$ ) for two days. The green crab mince was then removed from the refrigerator and mixed well in a plastic tote.

Moisture content of the mince was determined in triplicate using the draft drying procedure outlined below. Using a six quart KitchenAid bowl mixer Model KM25G0XWH (Dayton, OH) with paddle attachment, treatment ingredients were added and mixed on the 2 speed setting for seven minutes. While each batch was mixing, two small circular pieces of parchment paper were tared on a scale. One of the pieces of parchment paper was then placed on the bottom of the patty former Model 1404 (Univex; Salem, NH).

Once the batch was fully mixed, precisely  $100.0 (\pm 0.1)$  grams of the mixture were removed, placed on the bottom of the patty former and spread evenly using a rubber spatula. The second piece of parchment paper was placed on top of the green crab mixture and the lever of the patty former was depressed for ten seconds using a 6.6

pound weight to form the patty. The patty was then removed from the patty former along with the pre-tared pieces of parchment paper and was weighed to ensure a 100.0 gram patty. Each batch yielded two 100.0 gram patties. Patties were randomly placed on half sheet pans and refrigerated ( $5^{\circ}\text{C} \pm 1^{\circ}$ ) for 24 hours to allow the transglutaminase to take effect.

After 24 hours, the patties were removed from the refrigerator and the parchment paper was removed carefully. The sheet pans containing the patties were cooked in a  $150^{\circ}\text{C} (\pm 2^{\circ}\text{C})$  Blodgett Combination oven Model BC14E/AB (G.S. Blodgett Corp.; Burlington, VT) for 7.5 minutes, rotated, and cooked for an additional 7.5 minutes. The patties were removed from the oven and their internal temperatures were taken using a Type K Fisher Scientific Thermocouple (Pittsburgh, PA). The patties were cooled to room temperature under a fume hood and their post-cook weights were recorded.

#### Yield

Post cook % patty yields were calculated by dividing post-cook patty weights by pre-cook patty weights in grams and multiplying by 100.

#### Moisture Determination

Each batch was analyzed for total moisture in duplicate. Aluminum pans were labeled according to treatment and replication number. Pans were weighed on an analytical balance Model HR200 (A&D Co. Ltd.; Tokyo, Japan) to the nearest 0.001 g, and

pan weight was recorded. Samples were homogenized using a Black and Decker® Handy Chopper Plus (Household Products Inc.; Shelton, CT). The pans were tared and approximately 6 grams of homogenized sample was spread evenly onto the pan. The weight of each sample was recorded to the nearest 0.001 g. Each sample was placed in a 100° C Fisher Isotemp draft oven Model 350 (Fisher Scientific; Pittsburgh, PA) for at least 6 hours but no greater than 10 hours. The pans were removed from the oven and allowed a few minutes to cool. They were then re-weighed to the nearest 0.001 g and recorded. Total moisture content was analyzed based on AOAC method # 950.46 (AOAC, 2005). Moisture was determined using the following calculation:

$$\frac{(\text{pan wt.} + \text{sample wt.}) - (\text{pan} + \text{dry sample wt.})}{\text{sample wt.}} \times 100$$

#### Water-Holding Capacity

In each batch, one of the two patties was cut directly in half. The half patty was then cut again for access to the center. Four pieces of #1 Whatman filter paper were weighed to the nearest 0.0001 g. Approximately 2 grams of patty was cut directly from its center and placed on the pieces of Whatman filter paper. The paper was folded to fully cover the patty samples and placed into 50 mL centrifuge tubes. The samples were centrifuged for 15 minutes at 1000 x g using an Eppendorf centrifuge Model 5430 (Hamburg, Germany). The paper was removed from the centrifuge tubes and the sample pellet was removed. The paper, which now contains the water from the sample, was re-weighed to the nearest 0.0001 g. Samples were analyzed in duplicate and water-holding capacity was expressed as percentage of water retained with respect to the

water present in the patty before centrifugation. Water holding capacity was determined using the following calculation:

$$\frac{(\% \text{ moisture} \times 100) - ((\text{water wt. extracted from the sample} / \text{initial sample wt.}) \times 100)}{(\% \text{ moisture} \times 100) \times 100}$$

### Texture Profile Analysis

Patties were cut in half and measured for height with a Traceable® digital caliper Model 14-648-17 (Friendswood, TX). Using a TA-XT2i texture analyzer (Texture Technologies; Scarsdale, NY) patty halves were subjected to a double compression test at a speed of 50 mm min<sup>-1</sup> using a 25 N load cell by a 50 mm cylindrical plunger to 60 % of the original patty height. Each patty half was analyzed separately. Hardness in grams of force (g), adhesiveness in grams per second (g.s), springiness (dimensionless), cohesiveness (dimensionless), gumminess (dimensionless), chewiness (dimensionless) and resilience (dimensionless) were recorded. Hardness is the peak force of the first compression. Adhesiveness is represented as a negative force and represents the total force needed to pull the plunger away from the patty samples. Springiness is represented by how well the patties regain shape after the first compression. Cohesiveness is how well the patties withstand a second compression compared to the first. Resilience is defined by how quickly the patties regain their original position. Each batch was analyzed in duplicate equating to a total of six replicates per treatment.

### Color (L\*a\*b\*)

Green crab patties were subjected to colorimetric analysis using a LabScan XE Hunter Lab colorimeter (Hunter Associates Laboratory; Reston, VA) to determine L\*a\*b\* values. Patties were cut to fit the 2.5 inch clear glass sample cup with a black ring and disk, and placed on a pre-calibrated 2.5 inch sample port. L\*a\*b\* values were recorded using the computer software and each sample was turned  $\frac{1}{3}$  of a turn and read twice more. Analysis was completed in duplicate resulting in a total of six measurements per treatment.

### Crude Lipid Determination

Flat bottom flasks (~150 mL) were labeled and dried for approximately 10 minutes in a 100° C Fisher Isotemp draft oven Model 350 (Fisher Scientific; Pittsburgh, PA). They were removed from the oven, allowed to cool in a desiccator, and their weight was recorded on an analytical balance Model HR200 (A&D Co. Ltd.; Tokyo, Japan) to the nearest 0.001 g. Approximately 8 grams of crab sample were placed in a screw top glass bottle and sample weight was recorded to the nearest 0.001 g. Ten mL of 8.1 N hydrochloric acid (Fisher Scientific Inc.; Hampton, NH) were added to the bottles. The bottles were placed in an 85-90° C water bath for 90 minutes and the samples were shaken by hand at frequent intervals. They were then taken out of the water bath and allowed to chill on ice until cool to the touch. Seven mL of ethyl alcohol (Fisher Scientific Inc.; Hampton, NH) were added to each bottle and shaken by hand for approximately fifteen seconds. Twenty-five mL of ethyl ether (Fisher Scientific Inc.; Hampton, NH) were

then added to each bottle and shaken vigorously by hand for an additional one minute. Twenty-five mL of petroleum ether (Fisher Scientific Inc.; Hampton, NH) were then added to each bottle and shaken for another minute. The samples were allowed to let stand until a two phase separation occurred. The top phase was clear and contained the fat and ethers while the bottom phase contained all other components. The upper clear liquid was then transferred using a pipette into the pre-labeled and pre-weighed flat bottom flasks. The addition of ethyl ether and petroleum ether was repeated two more times with 15 mL of each. The upper clear liquid was transferred to the same flat bottom flasks for the last two extractions. The flat bottom flasks were then left under a fume hood overnight to allow the ether to evaporate leaving only the crude lipid content. The flat bottom flasks were dried in a 100° C Fisher Isotemp draft oven Model 350 (Fisher Scientific; Pittsburgh, PA) for 10 minutes. The flasks were then allowed to cool in a desiccator and the final weight was recorded to the nearest 0.001 g. Total crude lipid content was analyzed based on AOAC method # 922.96 (AOAC, 2005). Crude lipid content was determined on a wet weight basis using the following calculation:

$$\frac{((\text{flask} + \text{lipid wt.}) - \text{flask wt.})}{\text{sample wt.}} \times 100$$

#### Ash Determination

Patty samples were dried for 6-10 hours in a 100° C Fisher Isotemp draft oven Model 350 (Fisher Scientific; Pittsburgh, PA) and allowed to cool. Dried patty samples were crushed into a fine powder using a mortar and pestle. Scintillation vials were labeled using a diamond pen and weighed to the nearest 0.001 g. The vials were tared

and approximately 1 gram of sample was added to each scintillation vial, and the final weight was recorded. The samples were placed in a 550° C Thermolyne Model F-A1730 muffle oven (Dubque, IA) for 6 hours. The samples were allowed to cool and were re-weighed. Samples were analyzed in triplicate and total ash content was analyzed based on AOAC method # 938.08 (AOAC, 2005). Percent ash was determined on a dry weight basis (dwb) using the following calculation:

$$\frac{(\text{vial wt.} + \text{ash wt.}) - \text{vial wt.}}{\text{dry sample wt.}} \times 100$$

Percent ash was determined on a wet weight basis by using the following calculation:

$$\frac{(100 - \% \text{ average moisture content}) \times \% \text{ ash (dwb)}}{100}$$

#### Crude Protein Determination

Nitrogen content of ~100 mg dried patty samples was analyzed using the Elementar Rapid N III combustion nitrogen analyzer (Elementar Americas Inc.; Mount Laurel, NJ). Crude protein was determined by multiplying % nitrogen by a 6.25 conversion factor. Samples were analyzed in duplicate.

#### Statistical Analyses

Statistical differences among patty treatments for both experiments were evaluated using JMP 7.0.1 (SAS Institute Inc.; Cary, NC) statistical software using one-way analysis of variance (ANOVA) with a significance value of  $p \leq 0.05$ . Differences between means were evaluated using Fisher's least significant difference tests. In the



second experiment, a multi-way analysis of variance was conducted to evaluate the effects of transglutaminase, isolated soy protein and dried egg white additions for each dependent variable.

## Results and Discussion

### Yield

In experiment 1, average patty yields were 67.6 (C), 66.6 (T1), 66.0 (T2) and 66.4 (T4) percent (Figure 17). No significant differences in patty yields were detected among treatments based on one-way ANOVA.

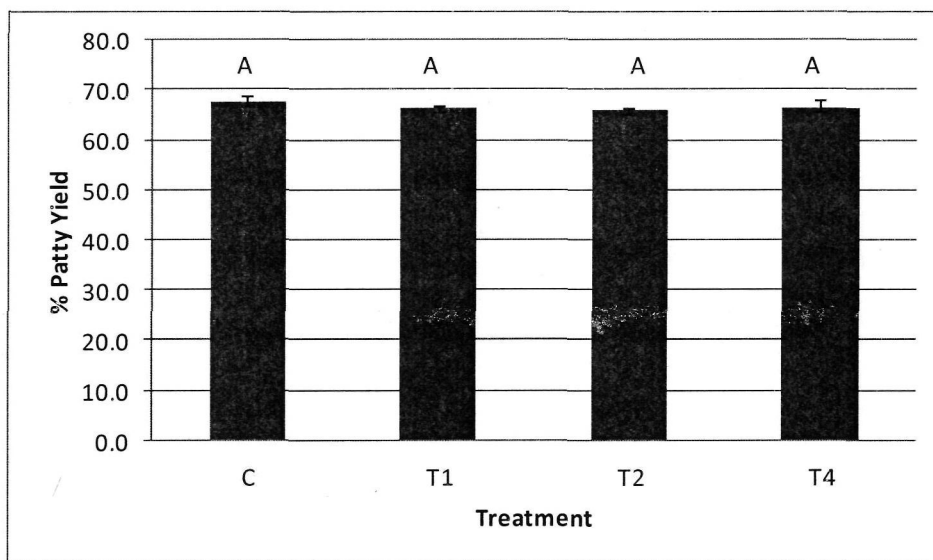


Figure 17 Mean patty yields (%) ( $\pm$  S.D) for experiment 1. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

In experiment 2, average patty yields were 66.1 (C), 77.0 (I), 73.8 (E), 76.3 (TI) and 75.7 (TE) percent (Figure 18). The additions of isolated soy protein and dried egg white significantly increased patty yields compared to the control. Patties with added proteins + transglutaminase did not have significantly different patty yields compared to patties with isolated soy protein and dried egg white alone.

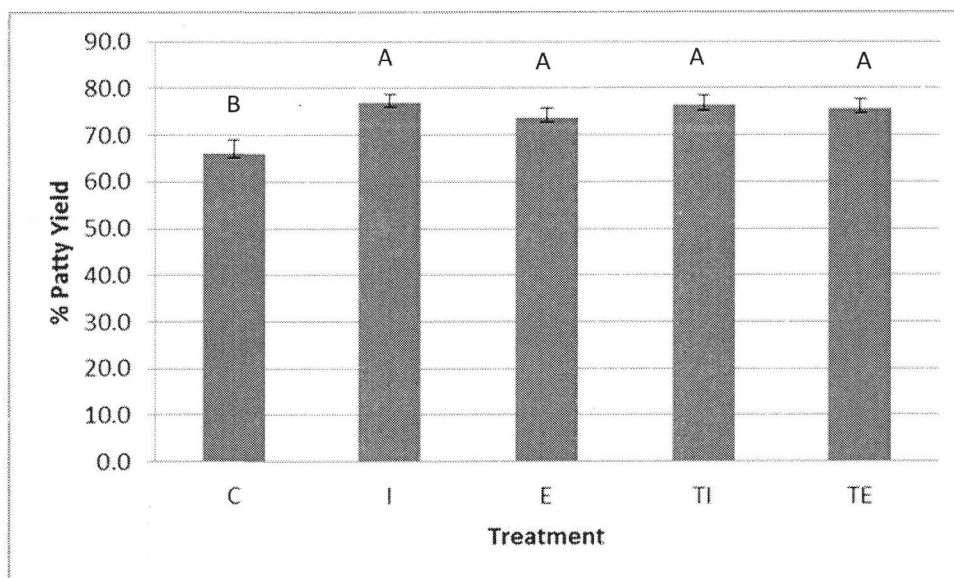


Figure 18 Mean patty yields (%) ( $\pm$  S.D) for experiment 2. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 % Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

Min and Green (2008) tested the effectiveness of microbial transglutaminase (0.05 - 0.7 %) and isolated soy protein (1.7 %) in raw patties made from the belly meat of channel catfish (*Ictalurus punctatus*). They reported that the addition of isolated soy protein significantly increased cooking yield compared to the control patties; however,

the yield of patties containing transglutaminase was significantly lower than that of the control (Min and Green, 2008). In this study, although transglutaminase additions did not significantly lower cook yield, there was a decrease in patty yield of 1.5 % between the control and 2 % transglutaminase treatments, which supports the research by Min and Green (2008). Additions of isolated soy protein and dried egg white significantly increased cook yield because these restructuring proteins were able to aggregate with other ingredients which may have improved the gel strength and water-holding capacity of green crab mince meat patties. Results from research conducted by Min and Green (2008) are similar to the results from this study.

#### Moisture Content

In experiment 1, average moisture contents were 61.0 (C), 59.5 (T1), 58.1 (T2) and 56.0 (T4) percent (Figure 19). Each treatment was shown to be significantly different ( $p \leq 0.05$ ) from one another based on one-way ANOVA. As the levels of transglutaminase increased, moisture content decreased.

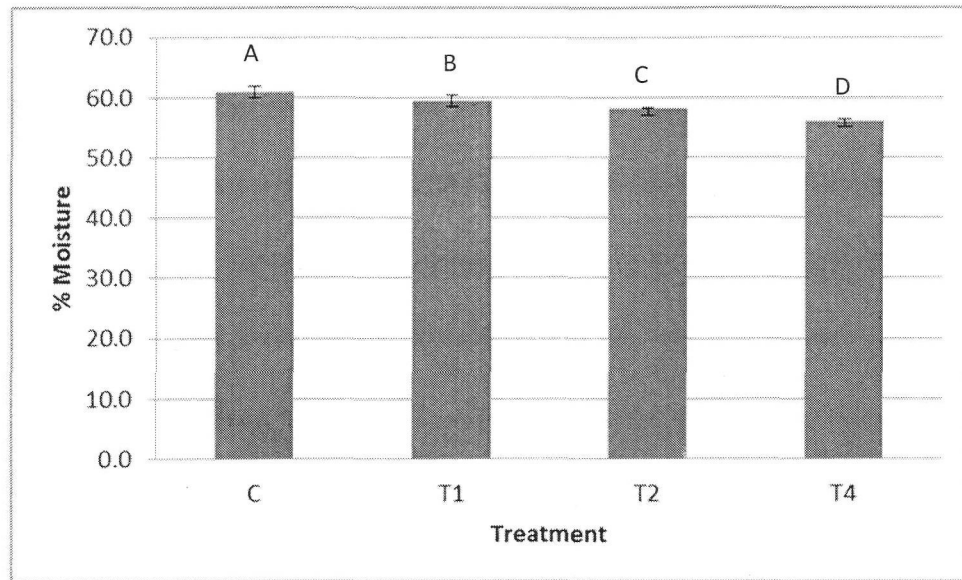


Figure 19 Mean moisture contents (%) ( $\pm$  S.D) for experiment 1. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

In experiment 2, average moisture contents were 59.6 (C), 60.8 (I), 59.6 (E), 58.4 (TI) and 58.1 (TE) percent (Figure 20). No significant differences were noticed among treatments based on one-way ANOVA.

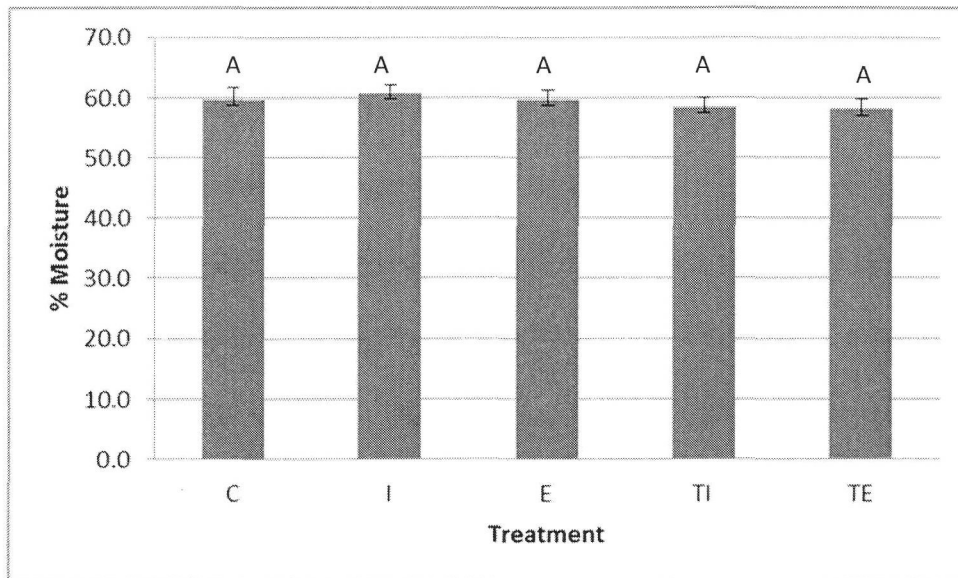


Figure 20 Mean moisture contents (%) ( $\pm$  S.D) for experiment 2.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 % Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

In both experiments, the moisture content of the initial crab mince used in the patty formulations was 75.5 percent. This equates to a range of 19.2 – 23.1 percent moisture loss after cooking among all treatments. A linear correlation existed between yield and moisture data with a 0.62 correlation coefficient ( $p < 0.04$ ), which indicated that as more moisture was lost during cooking, patty yield decreased. Treatments containing additions of Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation had significantly lower moisture contents; whereas, additions of isolated soy protein or dried egg white did not have the same effect. Increased transglutaminase additions may have caused more protein aggregation and a tighter protein network resulting in more moisture loss during cooking (Min and Green,

2008). Min and Green (2008) experienced moisture loss of 9.3 (control), 11.5 (0.05 – 0.7 % transglutaminase) and 6.9 (1.7 % isolated soy protein) percent during their study on channel catfish (*Ictalurus punctatus*) belly flap patties, and showed a significant difference among treatments. Min and Green (2008) experienced less moisture loss compared to this study because they were using a raw protein system which holds moisture more effectively than previously cooked mince which has less protein functionality due to denatured proteins.

#### Water-Holding Capacity

Water-holding capacity represents the amount of water lost under moderate centrifugal conditions and can be used as an indicator of protein functionality. In experiment 1, water-holding capacities were 52.7 (C), 52.1 (T1), 50.9 (T2) and 54.2 (T4) percent (Figure 21). No significant differences in water-holding capacities were detected among treatments based on one-way ANOVA. Variability existed in treatment T4 data which may have overestimated average water-holding capacity for that treatment. if there were less variability, the water-holding capacity would likely have decreased linearly with increased additions of transglutaminase.

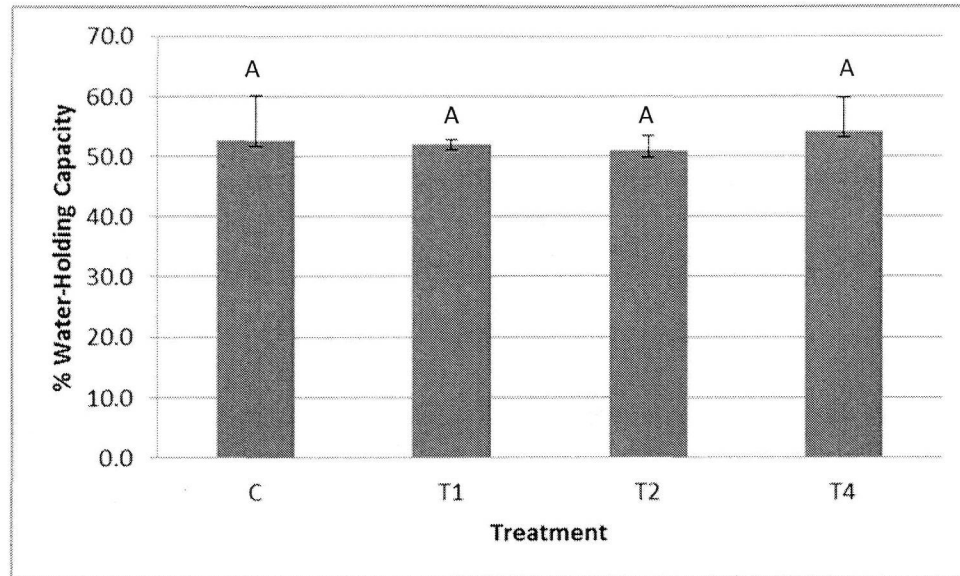


Figure 21 Mean water-holding capacities (%) ( $\pm$  S.D) for experiment 1. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

In experiment 2, average water-holding capacities were 48.7 (C), 53.3 (I), 59.7 (E), 57.4 (TI) and 61.3 (TE) percent (Figure 22). Treatments E, TI and TE were significantly different ( $p \leq 0.05$ ) from the control, whereas treatment I was not, based on one-way ANOVA. Patties with additions of isolated soy protein + transglutaminase and dried egg white + transglutaminase did not have significantly different water-holding capacities compared to patties with additions of isolated soy protein and dried egg white alone.

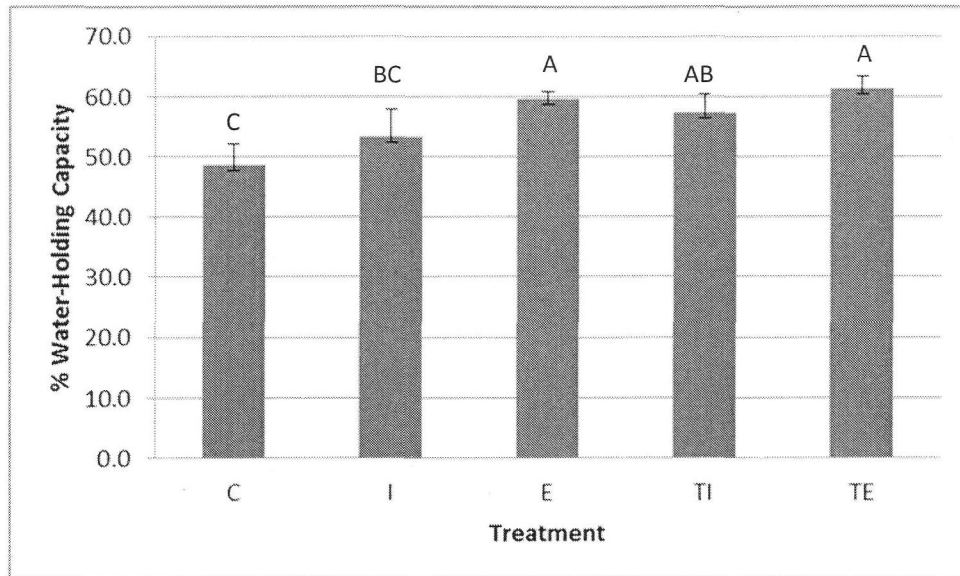


Figure 22 Mean water-holding capacities (%) ( $\pm$  S.D) for experiment 2. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 % Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

Baxter (2007) concluded similarly that additions of isolated soy protein in Jonah crab (*Cancer borealis*) mince meat gels did not significantly alter water-holding capacity. Results from Baxter (2007) showed an average water-holding capacity of Jonah crab (*Cancer borealis*) mince meat gels containing 5, 10 and 15 percent isolated soy protein to be 82.6 percent. The higher water-holding capacity seen by Baxter (2007) compared to this study is likely the result from using washed mince meat which may have increased functionality and water retention compared to unwashed mince.



### Texture Profile Analysis

Determining the texture of green crab mince patties is important in understanding consumer acceptability and processing conditions of the product. In experiment 1, patty hardness was 17335 (C), 16849 (T1), 15607 (T2) and 11042 (T4) grams of force (g) (Table 7). As levels of transglutaminase increased, patty hardness decreased. Treatment T4 was significantly ( $p \leq 0.05$ ) softer than the control treatment based on one-way ANOVA. Patty adhesiveness was -286 (C), -283 (T1), -316 (T2) and -139 (T4) grams per second (g.s) (Table 7), and shows treatment T4 was significantly less adhesive than the other treatments. This result indicates that as more transglutaminase was added to the patties, the less sticky the patties became. Patty springiness was 0.795 (C), 0.851 (T1), 0.841 (T2) and 0.745 (T4) (Table 7) with treatment T4 significantly less springy than the other treatments. Springiness is represented by how well the patties regain shape after the first compression. Patty cohesiveness was 0.403 (C), 0.463 (T1), 0.459 (T2) and 0.565 (T4) (Table 7). Treatment T4 was significantly more cohesive than the other treatments, and cohesiveness represents how well the patties withstand a second compression compared to the first. Patty gumminess was 7152 (C), 7828 (T1), 7186 (T2) and 6277 (T4) (Table 7). Patty chewiness was 5745 (C), 6665 (T1), 6048 (T2) and 4664 (T4) (Table 7). No significant differences were noticed between treatments for either gumminess or chewiness. Patty resilience was 0.169 (C), 0.197 (T1), 0.197 (T2) and 0.242 (T4) (Table 7). The control treatment was significantly less resilient than treatment T4. Resilience is defined by how quickly the patties regain their original position.

Table 7 Mean Texture Profile Analysis Values ( $\pm$  S.D) from Experiment 1

Treatment	Hardness (g)	Adhesive-ness (g.s)	Springi-ness	Cohesive-ness	Gummi-ness	Chewiness	Resilience
C	17335(a) ( $\pm$ 1723)	-286(b) ( $\pm$ 55)	0.80(a) ( $\pm$ 0.03)	0.40(b) ( $\pm$ 0.04)	7152(a) ( $\pm$ 924)	5745(a) ( $\pm$ 937)	0.17(b) ( $\pm$ 0.02)
T1	16849(a) ( $\pm$ 4840)	-283(b) ( $\pm$ 65)	0.85(a) ( $\pm$ 0.01)	0.46(b) ( $\pm$ 0.05)	7828(a) ( $\pm$ 2717)	6665(a) ( $\pm$ 2267)	0.20(ab) ( $\pm$ 0.02)
T2	15607(ab) ( $\pm$ 2304)	-316(b) ( $\pm$ 70)	0.84(a) ( $\pm$ 0.04)	0.46(b) ( $\pm$ 0.06)	7186(a) ( $\pm$ 1894)	6048(a) ( $\pm$ 1894)	0.20(ab) ( $\pm$ 0.02)
T4	11042(b) ( $\pm$ 773)	-139(a) ( $\pm$ 103)	0.74(b) ( $\pm$ 0.08)	0.57(a) ( $\pm$ 0.06)	6277(a) ( $\pm$ 772)	4664(a) ( $\pm$ 587)	0.24(a) ( $\pm$ 0.03)

Texture attribute values within columns not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

Overall, these results showed an increase in some of the textural attributes in patties which contained 1 and 2 % Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation, however values were not significantly different from control patties. Transglutaminase addition appeared to decrease the hardness, adhesiveness, springiness, gumminess and chewiness of the patties compared to the control. A stepwise decreasing trend in patty hardness was noticed with increased transglutaminase additions. Many of the textural attributes in treatment T4 were significantly different from other treatments which indicated that high levels of transglutaminase significantly changed the texture profile of green crab patties. A study by Ramírez et al. (2007) showed an increase in the mechanical properties of low salt striped mullet (*Mugil cephalus*) fish gels with transglutaminase additions. In addition, the mechanical properties of low-salt restructured silver carp (*Hypophthalmichthys molitrix*) gels were also increased by adding 3 g/kg of microbial transglutaminase (Uresti et al., 2004). Understanding the textural changes of green crab mince meat patties in

response to different levels of transglutaminase additions may be useful for further product development endeavors that include green crab mince meat.

In experiment 2, patty hardness was 16509 (C), 21630 (I), ND (no data) (E), 25950 (TI) and ND (TE) grams of force (g) (Table 8). Patties with the additions of isolated soy protein were significantly harder than the control treatment; however, the addition of the transglutaminase did not significantly ( $p \leq 0.05$ ) affect hardness. Patty adhesiveness was -241 (C), -309 (I), ND (E), -26 (TI) and ND (TE) grams per second (g.s) (Table 8). Patties with additions of transglutaminase showed a significant decrease in patty adhesiveness. No significant differences were noticed between treatment I and the control treatment. Patty springiness was 0.806 (C), 0.770 (I), ND (E), 0.911 (TI) and ND (TE) (Table 8). Patties with additions of transglutaminase showed a significant increase in patty springiness. No significant difference was noticed between treatment I and the control treatment. Patty cohesiveness was 0.440 (C), 0.436 (I), ND (E), 0.564 (TI) and ND (TE) (Table 8). Patty gumminess was 7295 (C), 9543 (I), ND (E), 14796 (TI) and ND (TE) (Table 8). No significant differences were seen between treatments for cohesiveness or gumminess. Patty chewiness was 5945 (C), 7331 (I), ND (E), 13388 (TI) and ND (TE) (Table 8). For chewiness, no significant difference was noticed between treatment I and the control treatment; however, additions of transglutaminase significantly increased patty chewiness. Patty resilience was 0.192 (C), 0.197 (I), ND (E) 0.274 (TI) and ND (TE) (Table 8). No significant difference in patty resilience was noticed between treatments. No data (ND) was recorded for patties containing dried egg white due to the patties being too hard for analysis.

Table 8 Mean Texture Profile Analysis Values ( $\pm$  S.D) from Experiment 2

Treatment	Hardness (g)	Adhesive-ness (g.s)	Springi-ness	Cohesive-ness	Gummi-ness	Chewi-ness	Resilience
C	16590(b) ( $\pm$ 1058)	-241(b) ( $\pm$ 9)	0.81(ab) ( $\pm$ 0.05)	0.44(a) ( $\pm$ 0.07)	7295(b) ( $\pm$ 1173)	5945(b) ( $\pm$ 1260)	0.19(a) ( $\pm$ 0.04)
I	21630(a) ( $\pm$ 1655)	-309(b) ( $\pm$ 100)	0.77(b) ( $\pm$ 0.01)	0.44(a) ( $\pm$ 0.04)	9543(b) ( $\pm$ 1436)	7331(b) ( $\pm$ 1194)	0.20(a) ( $\pm$ 0.03)
E	ND	ND	ND	ND	ND	ND	ND
TI	25950(a) ( $\pm$ 3569)	-26(a) ( $\pm$ 10)	0.91(a) ( $\pm$ 0.08)	0.56(a) ( $\pm$ 0.09)	14796(a) ( $\pm$ 3826)	13388(a) ( $\pm$ 2951)	0.27(a) ( $\pm$ 0.08)
TE	ND	ND	ND	ND	ND	ND	ND

Texture attribute values within columns not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. ND = No Data; C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 % Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

Overall, these results demonstrate that the addition of isolated soy protein did not affect the texture profile significantly in green crab mince meat patties. Only the hardness attribute showed a significant increase with a 5 % addition of isolated soy protein. This experiment has demonstrated the effectiveness of Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation in modifying the texture profile of green crab mince meat patties. Many of the texture attributes were unchanged with the addition of isolated soy protein alone; however, the combination of 5 % isolated soy protein and 2 % transglutaminase was shown to be most effective in causing significant effects in texture attributes. It is possible that the isolated soy protein increased the effectiveness of the transglutaminase by providing a greater amount of free amino acid residues and subsequently allowing the transglutaminase to form more protein bonds. Baxter (2007) performed a similar study on Jonah crab (*Cancer borealis*) gels using isolated soy protein and dried egg white additions. Egg white protein additions resulted in the hardest gels of all the additives, averaging 41.0 N (Baxter, 2007). The gels with

additions of isolated soy protein were significantly harder than the control gels, but were softer than the gels with added egg white protein by almost half (Baxter, 2007).

In this study, the addition of 5 % dried egg white resulted in a green crab patty product which was not acceptable for textural analysis. During cooking, the egg protein set and formed a very hard gel. The hardness of the dried egg white patties resulted in an 'error' message when using the texture analyzer. The plunger was restricted from pushing into the patty at 60 % of the patty height. In addition, semi-coagulated egg protein was noticed dripping from the patties during cooking which resulted in a loss of patty homogenization. Patties with egg white or transglutaminase additions were stickier and more difficult to work with. These negative effects may result in difficulty formulating value-added green crab mince meat products containing high levels of dried egg white or transglutaminase additions. Based on textural data and experimental observations, the additions of 5 % isolated soy protein and 2 % transglutaminase resulted in green crab mince patties with the most acceptable functional properties and were easiest to handle during preparation.

#### Color (L\*a\*b\*)

In experiment 1, average L\*a\*b\* values were 30.9, 7.9, 12.2 (C); 30.1, 8.1, 12.4 (T1); 30.7, 7.8, 12.5 (T2) and 31.5, 7.8, 12.1 (T4) (Figure 23). Average L\*a\*b\* values among all treatments were 30.8, 7.9 and 12.3. Treatment T1 had a significantly ( $p \leq 0.05$ ) higher a\* value indicating a redder color. Treatment T1 had a significantly ( $p \leq$

0.05) lower L\* value than treatment T4. No significant differences were noted among treatments for the b\* color value.

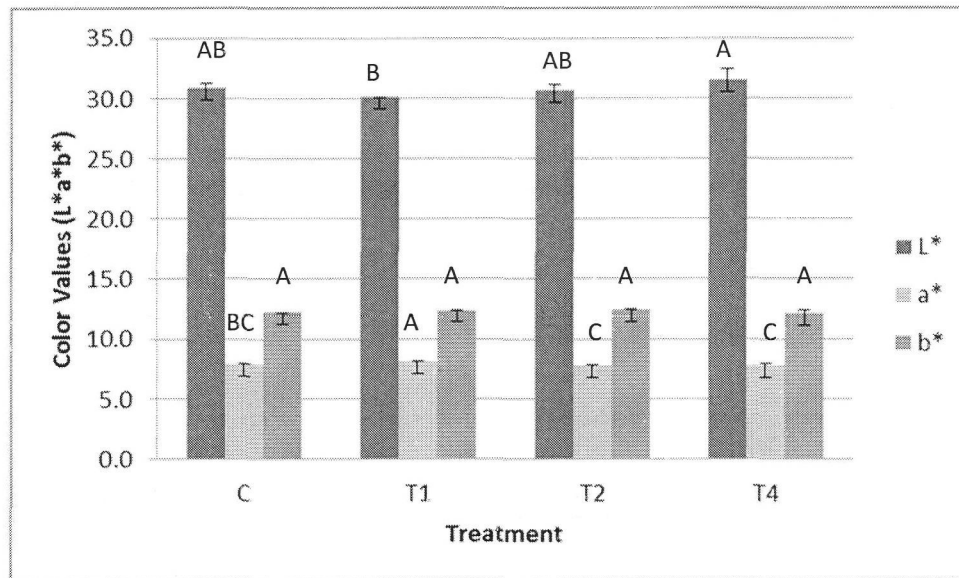


Figure 23 Mean color values ( $\pm$  S.D) for experiment 1.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

In experiment 2, average L\*a\*b\* values were 31.7, 7.6, 12.2 (C); 25.7, 6.5, 8.9 (I); 28.5, 6.7, 9.8 (E); 30.3, 7.0, 10.8 (TI) and 29.2, 5.9, 9.6 (TE) (Figure 24). Average L\*a\*b\* values among all treatments were 29.1, 6.7 and 10.3. Isolated soy protein and dried egg white treatments had significantly lower ( $p \leq 0.05$ ) L\* values compared to the control. This darker color is most likely due to an increase in Maillard browning reactions during cooking. Additions of isolated soy protein and dried egg white significantly reduced both a\* and b\* color values compared to the control. Patties with additions of

transglutaminase showed significant differences in color values compared to patties containing solely isolated soy protein or dried egg white additions (Figure 24).

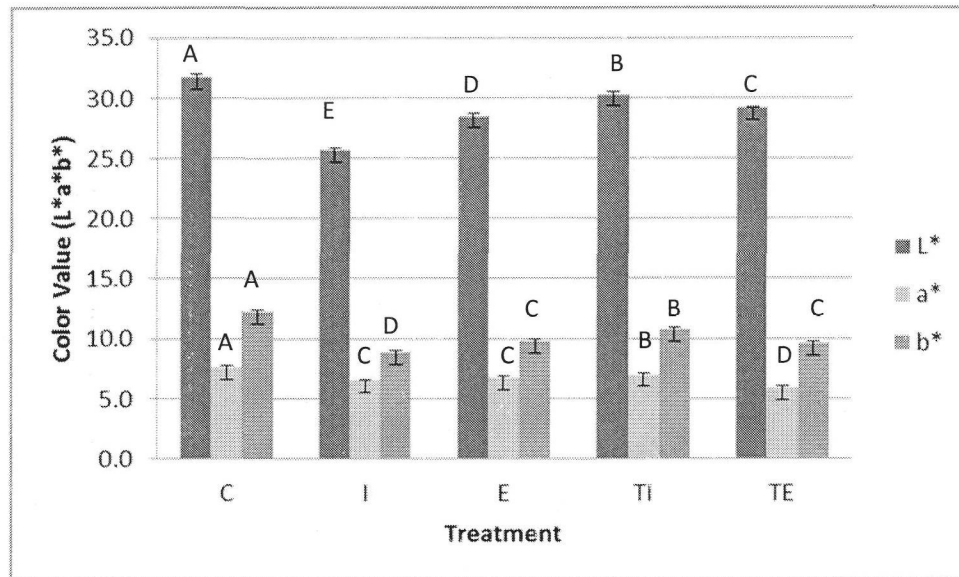


Figure 24 Mean color values ( $\pm$  S.D) for experiment 2.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 % Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

#### Crude Lipid Content

In experiment 1, average lipid content ranged from 2.7 – 2.8 % and specific lipid contents were 2.8 (C), 2.8 (T1), 2.8 (T2) and 2.7 (T4) percent (Figure 25). No significant differences ( $p \leq 0.05$ ) were detected among treatments based on one-way ANOVA.

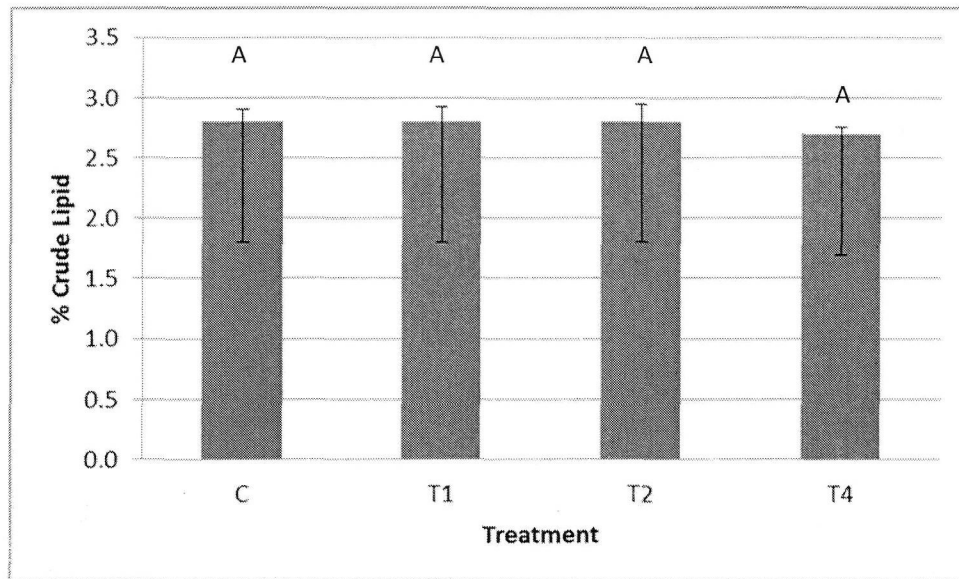


Figure 25 Mean crude lipid contents (%) ( $\pm$  S.D) for experiment 1. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

In experiment 2, average lipid contents were 2.9 (C), 2.4 (I), 2.1 (E), 2.6 (TI) and 2.2 (TE) percent (Figure 26). Treatments which contained isolated soy protein or dried egg white contained significantly ( $p \leq 0.05$ ) less crude lipid than the control treatment based on one-way ANOVA. A multi-way ANOVA showed that treatments containing added transglutaminase contained significantly more total lipid than treatments which contained isolated soy protein or dried egg white alone.



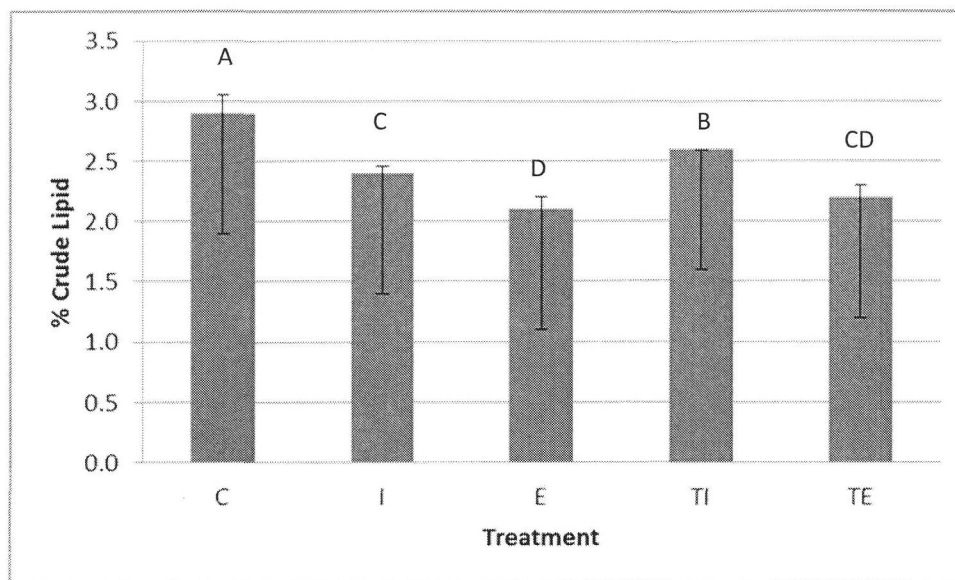


Figure 26 Mean crude lipid contents (%) ( $\pm$  S.D) for experiment 2.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 % Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

Green crab contains small amounts of lipids which are rich in omega-3 fatty acids and beneficial for heart health. The oxidation of lipids is a primary cause of quality deterioration in meat. Although green crab patties contain between 2-3 % total lipids, oxidative effects may take place during longer periods of frozen storage. Baxter (2007) showed that Jonah crab mince gels contain  $<1$  % crude lipid, although the gels were previously washed. In experiment 2, the increased lipid content of patties which contained added transglutaminase may have been due to the fact that Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation contained trace amounts of safflower oil.

### Ash Content

In experiment 1, ash contents ranged from 12.3 – 15.2 % and specifically were 12.3 (C), 13.0 (T1), 13.9 (T2) and 15.2 (T4) percent on a wet weight basis (Figure 27). Each treatment was shown to be significantly different ( $p \leq 0.05$ ) from one another based on one-way ANOVA. As the levels of transglutaminase increased, so did the percent ash content which may be attributed to the sodium chloride, trisodium phosphate and maltodextrin present in the Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation.

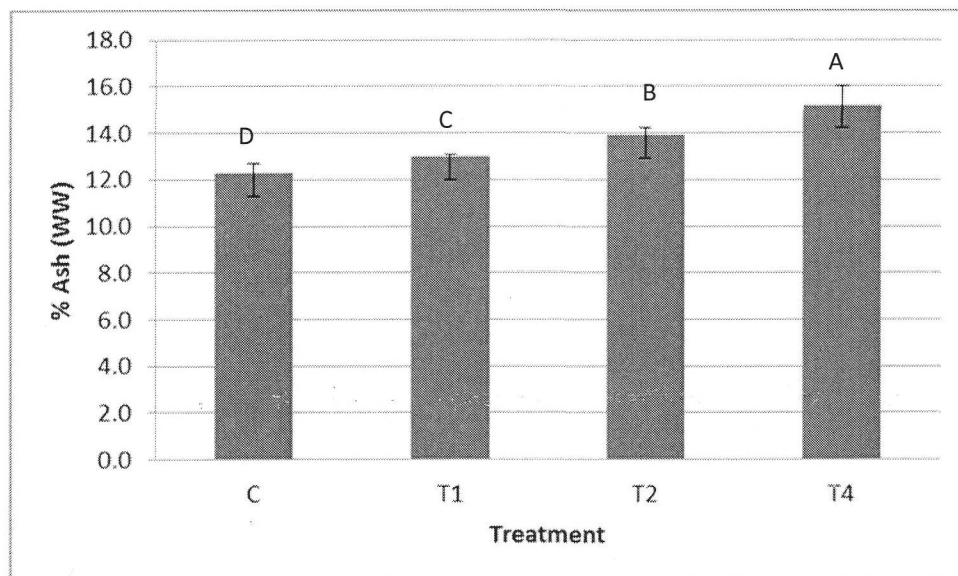


Figure 27 Mean ash contents (%) ( $\pm$  S.D) for experiment 1.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

In experiment 2, average ash contents were 12.6 (C), 10.7 (I), 10.9 (E), 11.8 (TI) and 12.2 (TE) percent on a wet weight basis (Figure 28). Treatments with isolated soy

protein and dried egg white additions contained significantly ( $p \leq 0.05$ ) less ash content than the control treatment based on one-way ANOVA which may be attributed to the isolated soy protein and dried egg white displacing the green crab mince, which contains a high proportion of shell particles that contribute to a high ash content.

Transglutaminase patties contained significantly more ash content than patties containing isolated soy protein or dried egg white additions alone based on multi-way ANOVA, which may be attributed to the sodium chloride, trisodium phosphate and maltodextrin present in the Activa® GS (Ajinomoto Co., Inc.; Kawasaki, Japan) transglutaminase preparation.

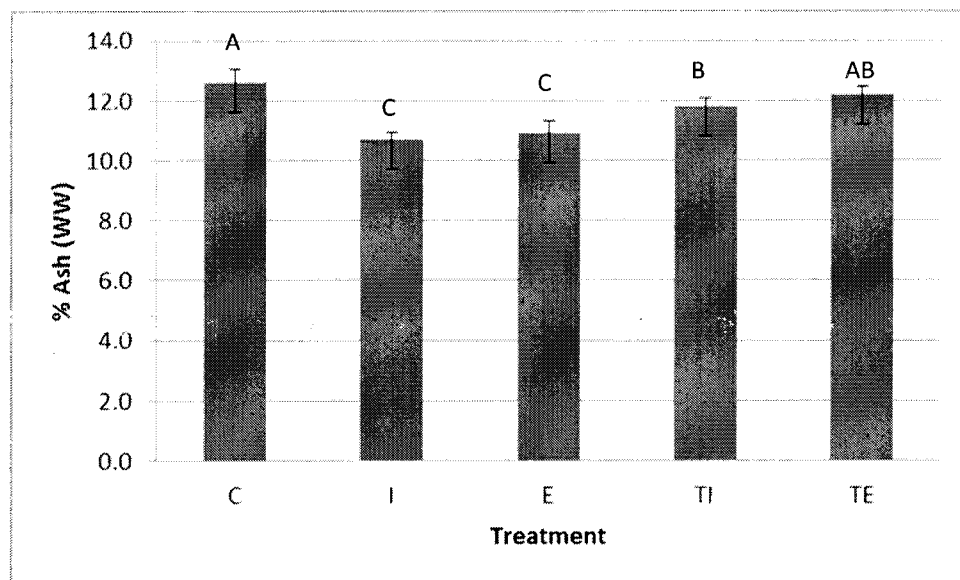


Figure 28 Mean ash contents (%) ( $\pm$  S.D) for experiment 2.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 % Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

Min and Green (2008) studied the effects of transglutaminase used at 0.05, 0.1, 0.2, 0.4 and 0.7 % concentrations, whey protein concentrate (1.7 %) and isolated soy protein (1.7 %) on the proximate composition of channel catfish patties. The ash content rose from 0.96 % (raw catfish mince) to 1.68 % (transglutaminase) and 1.79 % (isolated soy protein). This study showed a significantly higher ash content most likely due to the mechanical separation of whole green crab, which resulted in the incorporation of shell fragments, or 'shell-flour,' within the mince.

#### Crude Protein Content

In experiment 1, average protein content was 19.6 % for all treatments and specifically average protein contents were 19.4 (C), 19.6 (T1), 19.4 (T2) and 19.8 (T4) percent on a wet weight basis (Figure 29). No significant differences ( $p \leq 0.05$ ) in protein content were noticed among treatments based on one-way ANOVA. No significant correlations between protein content and other variables were noticed.

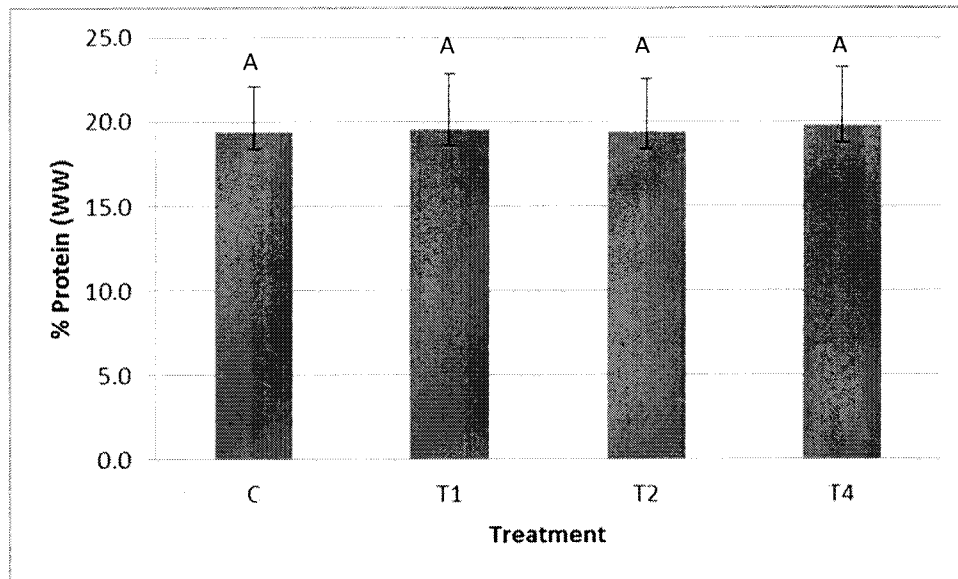


Figure 29 Mean protein contents (%) ( $\pm$  S.D) for experiment 1. Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; T1 = 1 % Transglutaminase; T2 = 2 % Transglutaminase; T4 = 4 % Transglutaminase

In experiment 2, average protein contents were 21.4 (C), 19.2 (I), 24.8 (E), 28.4 (TI) and 25.1 (TE) percent on a wet weight basis (Figure 30). Treatments containing transglutaminase + isolated soy protein were significantly ( $p \leq 0.05$ ) higher in protein content than the isolated soy protein and the control treatments based on one-way ANOVA. No significant correlations between protein content and other dependent variables were detected. Average protein content was 23.8 % for all treatments.

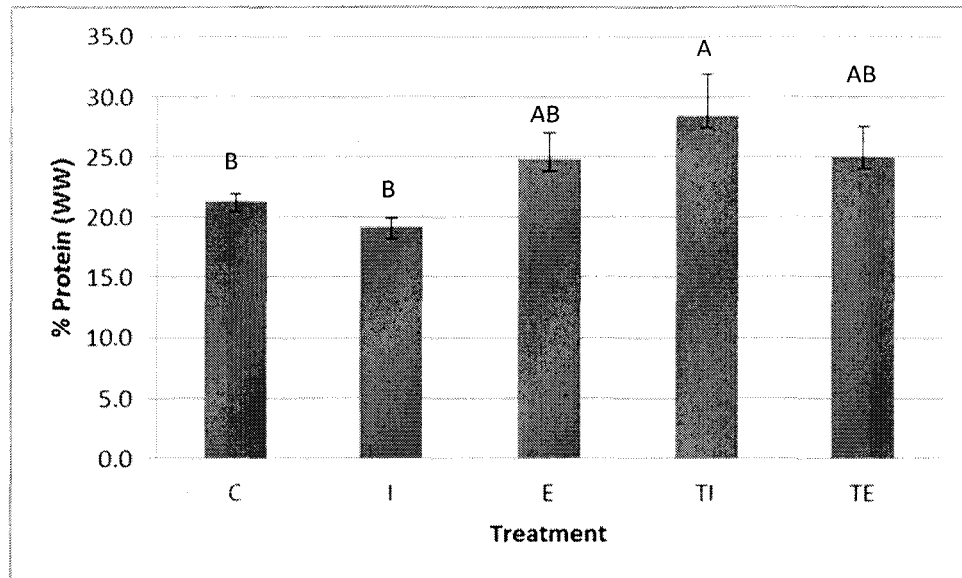


Figure 30 Mean protein contents (%) ( $\pm$  S.D) for experiment 2.

Treatments not sharing the same letter are significantly different ( $p \leq 0.05$ ) based on one-way ANOVA. C = Control; I = 5 % Isolated Soy Protein; E = 5 % Dried Egg White; TI = 2 % Transglutaminase + 5 % Isolated Soy Protein; TE = 2 % Transglutaminase + 5 % Dried Egg White

It is clear from both experiments that the addition of transglutaminase did not significantly increase the protein content of green crab mince patties. It was originally hypothesized that additions of isolated soy protein and dried egg white would increase the protein content of green crab mince patties, however there were no statistically significant differences compared to the control.

## Conclusions

This study focused on the effects of transglutaminase, isolated soy protein and dried egg white additions on the texture, color, cook yield, water-holding capacity and proximate composition of green crab mince meat patties. Increased additions of

transglutaminase resulted in a stepwise decrease in moisture content which resulted in a drier and less sticky green crab mince patty. Patty hardness also decreased with increased additions of transglutaminase, which was unexpected and may be a result of transglutaminase additions used at higher levels than in typical raw meat systems. In addition, the textural attributes of adhesiveness, springiness and cohesiveness were all significantly different ( $p \leq 0.05$ ) between treatment T4 and the other treatments, which indicate that high levels of transglutaminase may result in less desirable textural properties for product development purposes. Based on these results, it was determined that the addition of 2 % transglutaminase was most acceptable for subsequent experimentation.

Additions of isolated soy protein and dried egg white significantly ( $p \leq 0.05$ ) increased patty yield and water-holding capacity which supports the use of these additives in commercial processing operations.  $L^*$  and  $b^*$  color values were significantly ( $p \leq 0.05$ ) lower in treatments containing isolated soy protein or dried egg white, which is most likely a result of increased Maillard browning reactions during cooking. Patties containing isolated soy protein were significantly ( $p \leq 0.05$ ) harder than control patties; and patties containing 5 % dried egg white were too hard for instrumental texture analyses. The textural attributes of adhesiveness, springiness, gumminess and chewiness were all significantly different ( $p \leq 0.05$ ) between patties which contained isolated soy protein + transglutaminase and patties which contained isolated soy protein alone. The isolated soy protein may have increased the effectiveness of the transglutaminase by providing a greater amount of free amino acid residues and

subsequently allowing the transglutaminase to form more protein bonds. Results from data and experimental observations indicated that additions of 5 % isolated soy protein and 2 % transglutaminase resulted in green crab mince meat patties with the best functional properties and the most promise for further product development research to utilize green crab mince in value-added products.



## CHAPTER 5. OVERALL CONCLUSIONS

Green crab (*Carcinus maenas*) contains high quality protein and other beneficial nutrients which can be utilized through the development of processes that enhance the functional characteristics of the mince meat. Understanding the chemical composition of green crab mince as well as other quality factors is vital for subsequent processing and value-added production.

This study was performed in three parts and determined: how mechanical separation of green crab affected mince meat yield and quality; consumer acceptability of a green crab mince meat value-added product; and the physiochemical effects of adding restructuring additives to green crab mince patties.

High yield and low crude lipid content of mechanically processed green crab mince meat indicates its potential to be economically viable in commercial processing operations and retain its quality during extended periods of frozen storage. Proximate analyses showed green crab mince meat to contain quality protein and an ash content low enough for subsequent value-addition, which are factors in the marketability and processing of the mince. Low TVBN and APC values indicate that green crab mince meat is of good microbial quality. Although the mince extracted from small green crabs (<55 mm in carapace width) was darker in color than mince from their larger counterparts, mechanical processing of small green crabs was more feasible. In addition, mechanical processing difficulties at the 0.180 breaker bar setting indicate that a tighter grinding cavity is preferable. Overall results indicate that small green crabs which were

mechanically processed at a 0.000 breaker bar setting, regardless of thermal treatment, provided mince meat which contained the highest yield and were easiest to process.

A successful green crab mince and vegetable empanada was developed and statistical analysis indicated that empanadas containing 30 and 50 % mince meat were significantly ( $p \leq 0.05$ ) more liked in the attributes of filling appearance, flavor and overall acceptability compared to the 70 % empanadas. Panelist comments indicated that a sauce may be beneficial in providing a moister texture and enhancing the crab flavor of the green crab mince empanadas. Many of the panelists would 'probably or definitely buy' green crab and vegetable empanadas if they were available locally. Overall attribute scores approaching 'like moderately' (i.e. hedonic score of 7) for the 30 and 50 % empanadas, and strong willingness to purchase results indicate promise for green crab mince empanadas, however further product development is necessary to improve consumer acceptability of this novel product.

The use of transglutaminase to bind green crab mince in the formation of patties was not as effective as originally hypothesized. There was a significant ( $p \leq 0.05$ ) decrease in moisture content and patty hardness, and a non-significant decrease in yield as additions of transglutaminase increased. These factors lead to the conclusion that transglutaminase is not an acceptable additive in modifying the functional properties of pre-cooked green crab mince and may be better suited in raw meat systems. Additions of isolated soy protein and dried egg white resulted in more significant changes in functionality than transglutaminase alone, although the combination of 5 % isolated soy protein and 2 % transglutaminase appeared to be most effective in changing the

functional properties of green crab mince to produce a model patty based on textural and proximate analyses.

Future value-added studies utilizing green crab mince meat should investigate further the functional capabilities of mechanically processed green crab mince. It may be important to understand how to further modify the textual properties and flavor profile of green crab mince. The commercial production of value-added green crab mince meat products can help establish a commercially viable green crab fishery which would help alleviate negative consequences of green crab predation.

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## APPENDIX A

### CRAB AND VEGETABLE EMPANADA RECIPE

*Prep Time: 30-45 min*

#### Ingredients

Vegetable Oil (For Frying)	As needed
----------------------------	-----------

#### *For Dough:*

AP Flour	156 g
Salt	4 g
Double Acting Baking Powder	2 g
Vegetable Shortening	40 g
Water (Warm)	65 g

#### *For Filling:*

*(sample 1/30%)(sample 2/50%)(sample 3/70%)*

Crab Mince	54 g	126.4 g	295 g
Spanish Onion (Diced)	38 g	" "	" "
Corn Kernels (Fresh)	50 g	" "	" "
Red Pepper (Diced)	38 g	" "	" "
Thyme (Dried)	0.2 g	" "	" "
Cayenne Pepper (Dried)	0.2 g	" "	" "
Water	As needed	" "	" "

#### Method of Preparation

- 1.) In a sauté pan, add a small amount of vegetable oil and add the onion, red pepper and corn and cook on low-medium heat until vegetables begin to caramelize. Add water if needed. Add the thyme and cayenne pepper.
- 2.) In a bowl, mix the flour, baking powder, and salt together.
- 3.) Cream in vegetable shortening to dry ingredients until the mixture has a sandy resemblance.
- 4.) In an electric mixer using a dough hook or paddle, add the warm water and slowly add the dry ingredients to the water while mixing on low speed. Do not over mix the dough. Let the dough rest for 10 minutes.

- 5.) Add the appropriate amount of crab mince to the sauté pan with the vegetables and cook on low heat until the crab mince loses most of its water.
- 6.) On a floured surface, use a rolling pin to roll the dough ball out to a 0.5 mm thickness. The dough should almost be translucent. If it is translucent, the dough is too thin.
- 7.) Using a 4 ½ in. diameter cookie cutter, press out dough circles from the rolled out dough. The dough circles should weigh between 10-12 g.
- 8.) Re-form the extra dough, roll it back out to 1 mm thickness and cut out more 4 ½ in. dough circles.
- 9.) Place ~ 7.4 g of filling in the center of a dough circle.
- 10.) Pull one end of the filled dough circle over and press the edges together gently. Push gently down on the empanada to displace air from the filling before completely sealing.
- 11.) Further press the edges of the filled empanada together to form a solid seal.
- 12.) Repeat steps 9 – 11 for the remaining ingredients.
- 13.) Shallow fry empanadas in vegetable oil on low-medium heat for ~ 6 minutes on the first side and ~ 4 minutes on the other side or until light golden brown.

**APPENDIX B**

**CRAB AND VEGETABLE EMPANADA FLYER**

**Volunteers are needed to evaluate crab and vegetable empanadas.**



**Consumer Testing Center**

**168 Hitchner Hall Wednesday, February  
10th 9am - 2pm**

**Volunteers will receive a 120 minute phone card or a small snack.**

**This session will not last more than 20 minutes.**

**If you have allergies to wheat, soy or seafood, please do not participate.**

**For more information please e-mail Joseph Galetti on firstclass.**

## **APPENDIX C**

### **INFORMED CONSENT FORM**

Dear Participant,

If you are at least 18 years old and like to eat crab, you are invited to participate in a Master's thesis project being conducted by Joseph Galetti, a Food Science & Human Nutrition graduate student at the University of Maine, Orono. The purpose of this project is to evaluate consumer opinions about fried crab and vegetable empanadas. **If you have any allergies or sensitivities to seafood, soy, or wheat; or have never eaten these foods, we ask that you do not participate.**

#### **What Will You Be Asked to Do?**

- We will ask you some questions about yourself and your food purchasing habits.
- Next, you will be served three crab and vegetable empanada samples, and you will be asked questions regarding their acceptability.
- Lastly, we will ask you how we can improve the empanadas and if you would be willing to purchase them.
- Testing should last no more than 20 minutes.

#### **Risks**

Other than your time and inconvenience, there are no risks from participating.

#### **Benefits**

While this study will have little direct benefit to you, your participation may help create a commercial fishery for green crabs.

#### **Compensation**

You will receive a small gift such as a 120-minute phone card or a small snack if you complete this study.

#### **Confidentiality**

This study is anonymous. There will be no records directly linking you to the data that is collected on the computer. Records will not be held for more than 18 months after the completion of the study and will be kept in a locked room.

#### **Voluntary**

Your participation in this study is voluntary. If you choose to take part in this study, you may stop at any time during the study. However, if you do not finish the study, you will not be given a gift. You may skip any questions you do not wish to answer.

#### **Contact**

Questions regarding the empanada research can be directed to Joseph Galetti, 5735 Hitchner Hall, 581-6691, joseph.galetti@maine.edu or; Denise Skonberg, 104 Hitchner Hall, 581-1639, denise.skonberg@maine.edu.

If you have any questions about your rights as a research participant, please contact Gayle Jones, Assistant to the Protection of Human Subjects Review Board, 581-1498, gayle.jones@umit.maine.edu.

Thank you for your time and assistance.

**APPENDIX D**  
**CRAB AND VEGETABLE EMPANADA QUESTIONNAIRE**

First, please tell us about yourself. Any information that you provide will be held confidential. Once you have answered these questions, please click on the hand at the bottom of the computer screen to begin the empanada evaluations. Please evaluate the samples in the order shown in the upper blue box of your computer screen. Take a sip of water between each sample. There are 3 samples.

**What is your gender?**

Male                      Female

**What is your age?**

18-----90

**How often do you consume crab or products containing crab?**

[daily] [weekly] [monthly] [every few months] [once per year]  
[less than once per year] [never]

**In what form do you most often consume crab?**

[whole crabs] [soft-shell] [chowder/soup] [dumplings] [crab cakes]  
[seafood/crab salad] [dip] [fried appetizers] [other]\_\_\_\_\_

**In what setting do you most often consume crab products?**

[upscale restaurant] [family restaurant] [seafood restaurant] [take out/fast food] [outdoor event] [home] [other]\_\_\_\_\_

**What is your most preferred type of food?**

[American] [Chinese] [Japanese] [Middle Eastern] [Italian] [African] [Indian]  
[Hispanic] [French] [Creole] [Korean] [Mexican] [other]\_\_\_\_\_

---

**Please use the following scale to tell us how you like or dislike each sample. Please rate each sample completely before tasting the next sample.**

**How would you rate the appearance of the empanada?**

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

**How would you rate the appearance of the filling?**

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

**How would you rate the texture of the empanada?**

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

**How would you rate the flavor of the empanada?**

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

**How would you rate this empanada overall?**

1	2	3	4	5	6	7	8	9
Dislike Extremely	Dislike Very Much	Dislike Moderately	Dislike Slightly	Neither Like or Dislike	Like Slightly	Like Moderately	Like Very Much	Like Extremely

---

*(sample 2)*

---

*(sample 3)*

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**What improvements would you make to the empanadas you just tasted?**

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**If a product similar to the empanadas you just tasted was available to you locally, how likely would you be to purchase it?**

[Definitely would not buy] [Probably would not buy] [Probably would buy]  
[Definitely would buy]

**Additional comments?**

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**Your participation in this test has been greatly appreciated. Please raise your window slightly to let us now that you are done.**



## **BIOGRAPHY OF THE AUTHOR**

Joseph A. Galetti was born in Quincy, Massachusetts in July of 1983. He was raised in Plymouth, Massachusetts and graduated from Plymouth North High School in 2001. Joseph attended Cape Cod Community College for a period of 2 years where he studied Liberal Arts. He then attended Johnson & Wales University where he received an Associate's degree in Culinary Arts in 2004 and a Bachelor's degree in Culinary/Nutrition in 2006. While attending Johnson & Wales University, Joseph was employed by the Cranebrook Restaurant and Tea Room where he was able to further develop his culinary skill set. He then went on to work for Raw Seafoods Inc. and Decas Cranberry Products Inc. where he developed skills in food quality assurance, product development and leadership. Joseph enrolled in the Department of Food Science and Human Nutrition at the University of Maine in 2008 to work on his Master's degree in Food Science and Human Nutrition. Upon successful completion of the requirements for his Master's degree, Joseph will be working towards a Doctor of Philosophy degree in Food and Nutritional Sciences at the University of Maine. He is a candidate for the Master of Science degree in Food Science and Human Nutrition from the University of Maine in December, 2010.